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METABOLIC STUDIES IN A CASE OF OSTEITIS DEFORMANS*

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OSTEITIS deformans was first described by Sir James Paget of London in 1876. Although uncommon, it can hardly be regarded as a rare disease. In 1915, Da Costa *et al.* (1) reviewed the literature and found 213 cases, including 5 amongst their own records. Since then, the literature has greatly increased; there are to date 245 additional references. In 1925, Lewin (2) stated there were about 251 cases in the literature and on this basis Van Hazel and Andrews (3) concluded that the disease was rare. Since there were 213 cases prior to 1915, it would appear, according to Lewin's observation, that 38 cases only were reported between 1915 and 1925. As a matter of fact, the writer found 115, bringing the total number to 328 to the end of 1924. Since then 206 other cases have been reported, making a total of 534 to date. That the incidence of this disease is probably still greater is suggested from the rate of increase with the more general use of X-Rays. As is well known, X-Rays afford practically the only means of detecting this disease in its very early stages. With still more general use of this laboratory procedure, it appears reasonable that the incidence of this disease will probably be found to be still greater.

From the available literature, it is difficult to arrive at the true age and sex incidences, because these factors have received relatively little consideration in a large number of reports. A common practice appears to have been to report one case in detail and mention incidentally only the number of other cases met with. However, from the available data, the ages ranged from 6 to 89 years; the average was 56.8 years. There appeared to be more males than females; the ratio was about 1.5 to 1.

The pathology of this disease has frequently been dealt with in detail and, for purposes of brevity, will not be discussed here, except for relevant facts in the interpretation of our data. This communication is concerned

particularly with metabolic findings. Considering the number of reports, little consideration has been given to the metabolism of this disease. For example, though the intimate relationship between calcium, phosphorus and bone metabolism has been recognized for some time, only sixteen reports mention blood calcium studies and nine record blood phosphorus. Determinations of metabolic balances with respect to the inorganic elements are still fewer in number and the majority of them are relatively incomplete. For example, a few deal with calcium balance only and though others record calcium or phosphorus data, or both, no mention is made of sulfur. As Da Costa *et al.* point out, the changes in sulphur metabolism may be profound. The first and the most thorough investigation with regard to this phase of the disease was that of Da Costa, Funk, Bergeim and Hawk (1). More recently Cuthbertson reported balances of calcium, magnesium, phosphorus, sulphur and nitrogen (4). Both Da Costa *et al.* and Cuthbertson record essentially the same phenomena, namely, retention of calcium, magnesium and phosphorus and loss of sulfur. These findings appear to have been generally overlooked. Amongst the 245 new references, 28 only refer to the work of Da Costa *et al.* and such standard works as Sherman's Chemistry of Food and Nutrition, Lusk's Science of Nutrition and the most recent comprehensive work on "Quantitative Clinical Chemistry" (Peters and Van Slyke) make no mention of it. An opportunity was afforded for the investigation of the metabolism of a case met recently in the wards of The Montreal General Hospital and the purpose of this communication is to record our results. This patient was admitted into the Medical service of Dr. C. P. Howard, to whom I am very much indebted for his coöperation. The clinical details were, briefly, as follows:

Case History

A male, age 42 years, Hosp. No. 454/31, was admitted to this hospital on January 24th, 1931, complaining of pain in the chest, cough and "thickness of bones." The family history was irrelevant. The past history was also irrelevant, with the exception of "rheumatic fever" in 1913 and "frequent colds."

The present illness dates back to August, 1930, when weakness was first noticed. This was followed by loss of weight and pain in the chest. Since the beginning of the present illness he lost about twenty pounds.

The physical findings were negative, with the exception of some pyorrhoea and dental caries, chronic tonsillitis and hypertrophy of the heart with a systolic murmur at the mitral area, the residual of rheumatic heart disease. The essential skeletal features were lordosis in the dorsal lumbar region, a hydrocephalic type of skull, moderate kyphosis and bowing of the legs. The laboratory findings were as follows:—

Blood:	Red cells	4,980,000
	White cells	6,500
	Haemoglobin	88 per cent

Differential:—polymorphonuclear leucocytes	63	per cent
lymphocytes	18	" "
eosinophiles	4	" "
monocytes	10	" "
myelocytes	2	" "
basket cells	3	" "
Wassermann	negative.	

Urine: Clear: S.G. 1025: very faint trace of albumen; no sugar: no acetone bodies: occasional hyaline and granular cast and an average of 10 pus cells per high power field: pH 6.1

Blood Chemistry: Urea nitrogen	13	mgms. per 100 cc.
Creatinine	1.62	" " " "
Uric acid	3.47	" " " "
Calcium (serum)	9.2	" " " "
Phosphorus (inorganic)	3.84	" " " "
pH	7.41	" " " "
CO_2 combining power (plasma)	58.5	vol. per cent.
Sugar	0.117	per cent.

Blood Sugar Time Curve: In the fasting state the urine was free of sugar and acetone bodies and the blood sugar was normal, namely, 0.117 per cent. One hundred grams of glucose were then given by mouth with the following results:

	Blood sugar	Urine sugar
30 minutes after ingestion	0.200 per cent	0
60 " " "	0.169 " "	0
120 " " "	0.125 " "	0
150 " " "	0.100 " "	0

Electro-Cardiograph: Left ventricular preponderance with evidence of myocardial damage.

Basal Metabolic rate: +6 per cent.

X-rays: The chest showed evidence of a low grade non-tuberculous infection. The heart appeared normal. The findings in the bones were as follows:

Both femora showed increased diameter and loss of differentiation between the medulla and cortex and striation characteristic of Paget's disease. There was no evidence of excess calcification.

The left humerus was normal. The right humerus showed increased diameter, chiefly confined to the sub-periosteal region. The medulla and cortex presented a striated appearance with scattered areas of rarefaction. A similar change was noted in the tuberosity.

Similar changes to that found in the right humerus were also noted in the eighth rib.

There was some rarefaction of the bone at the lower end of the right tibia, involving the medulla and cortex, but more marked in the cortex toward the anterior surface of the bone. There was no marked calcification.

BALANCE OF INORGANIC ELEMENTS

The procedures (food, nursing, chemical methods, etc.) followed in our case conformed to our routine in such studies. As there is, perhaps, no other metabolism experiment in which more errors are likely to occur and nullify the value of data, an outline of this routine will be given briefly.

NURSING

In spite of the greatest care possible on the part of nurses, even in the best of organized general hospital wards, there are a number of possible sources of error which may readily nullify results. In our experience, at least, a common source of error is carelessness on the part of orderlies in the transmission of food from the diet kitchen to the patient, or of specimens of urine and feces from the patient to the laboratory. Such errors may, unfortunately, never be detected and lead to publication of erroneous conclusions. As the data upon which they are based are faulty, they

confuse rather than enlighten when reference is made to them in future studies. In our routine, when a metabolic balance is to be obtained, the experimental subject is isolated from all other patients in the ward. Special day and night nurses are assigned who have no other duties but the care of the experimental subject. The patient is, thus, under *constant* observation. Orderlies are not allowed near the patient; the special nurses bring the food and return residues, if any, to the diet kitchen. These nurses are also responsible for the collection of urine and feces and their transportation to the laboratory. A urine refrigerator is kept at the patient's bedside.[1] There is, thus, no opportunity of specimens being mixed with those of other patients in the ward. Feces are collected in ordinary bed-pans and delivered *undisturbed* to the metabolism laboratory where the total weight of feces and pan is immediately obtained. A portion of feces is then taken for analysis, the pan is washed and the clean pan, after having been weighed, is returned to the ward. Carmine is used for partition of feces. To ensure collection of *exactly* 24-hour specimens of urine, the patient is given a drink of water every morning about one hour before the last specimen is to be collected, in order to promote a mild polyuria. When pans or urinals are necessary for collection of urine, they are rinsed with water when the urine is transferred to the jar in the refrigerator and the rinsing is added to the specimen. Creatinine determinations are used as a further check of the quantitative collections.

Volumes of urine and weights of feces are recorded immediately after the specimens are obtained. The specimens are then divided into two parts, as a protective measure against loss due to accidental breakage of container, etc.

MEASUREMENT OF FOOD INTAKE

Judging from the literature, it is not an uncommon practice, when determining metabolic balances, to make use of available food tables for calculations of food intake, though elaborate precautions may be taken otherwise with the remaining procedures. The possible sources of error inherent in such tables with food constituents other than the inorganic elements, were previously referred to (5). This criticism applies still more to the inorganic elements. The marked discrepancies possible between actual and assumed compositions are strikingly demonstrated when data of such tables are compared with those obtained by analyses of food materials actually used in a given experiment. As Sherman's tables (6) are most frequently employed, it may here be observed that the values recorded represent averages of whatever reasonable looking data the author was able to find at the time.[2] In some cases the averages were results of many determinations, while in others, the numbers were small. It may, therefore, be observed, that the statistical principle underlying the use of averages is that the arithmetical mean of a *large* series of observed values is the most probable value of the quantity measured;[3] the smaller the number of observations from which an average is calculated, the less is the significance which can be attached to that average. However, the author of these tables never intended that they should be used for such purposes. The purpose of compiling the data given was to afford approximate evaluations of intake when diets contain *many* articles of food and individuals are exposed to them over *long periods* of time. Under these conditions, there should be, as the author puts it, "a fair statistical probability of errors offsetting each other." In *short period* experiments, with use of a *small* number of articles of food, conclusions drawn from data suggesting loss or retention of the different elements are justified only when the chemical analyses include not only urine and feces, but, also, the different

[1] These refrigerators have been in use in this hospital during the last ten years and have proven highly satisfactory. They consist of wooden boxes 8×12×12 inches, lined with zinc or copper, and are partitioned in the middle. The jar containing a preservative rests in one section, while the other section contains ice. The cover is also lined with zinc or copper. They are not unsightly though, because of the number of diabetics, cardio-renal cases, etc., ten or more may be seen frequently in one ward.

[2] Personal communication.

[3] This is, of course, true only for a limited type, the symmetrical, of frequency distribution.

food materials ingested. In such experiments, repeated observations (unpublished data) have shown that differences of twenty-five per cent may very frequently be encountered between actual and assumed food values; and differences of as much as fifty per cent and more are not uncommon.

Chemical Methods

We do not use any of the micro-chemical methods in such studies, tempting as they may be. A disadvantage of macro-methods is that they require large amounts of material and are time consuming. However, in such experiments, the amounts of material available are quite large and as probable errors increase with the smallness of the amount of material used, micro-methods have no place in such procedures. An additional contra-indication is the fact, repeatedly noted, that these micro-methods, quantitative as they may be from a theoretical point of view, are not so practical, unless very exacting attention is paid to minute details. As the latter are time consuming, such methods offer no advantages over the standard macro procedures. In these studies all methods were gravimetric; calcium and magnesium were determined by McCrudden's procedures; calcium was isolated as the oxalate, the latter was then ignited and the calcium gravimetrically determined as calcium oxide; magnesium was isolated from the filtrates of the calcium determinations, after destruction of the organic matter, as magnesium ammonium phosphate; the latter was then ignited and the magnesium gravimetrically estimated as the pyrophosphate. For sodium and potassium estimations, the organic matter of the materials was oxidized according to the Neumann procedure. The sodium and potassium were then isolated as chlorides, converted into their respective platinic chlorides and their separation was based upon the practical insolubility of potassium-chloro-platinate in strong alcohol. Total sulfur in the urine was estimated by Benedict's method of oxidizing the organic matter with copper nitrate and potassium chlorate and gravimetrically determining the sulfur as barium sulfate. For sulfur of food and feces, the more recent procedures of Stockholm and Koch (7) were made use of. Phosphorus was determined by oxidizing the organic matter of the materials by the Neumann process. The phosphorus was then isolated as a phosphomolybdate and converted into magnesium ammonium phosphate. The latter was then ignited and the magnesium determined gravimetrically as the pyrophosphate. Chlorine was estimated by oxidizing the organic matter with sodium peroxide followed by precipitation of the halogen with silver nitrate and gravimetric determination of the silver chloride formed. All nitrogen determinations were made by the macro-Kjeldhal technic. The above procedures are all standard and, as details are available in a variety of works on quantitative chemistry, for purposes of brevity, no descriptions will be given here.

Diets

The diet in this case was liberal with respect to its carbohydrate, fat, protein and caloric content and composition of inorganic elements. As a matter of fact, according to available maintenance requirement data, excess quantities were largely used. The possible influence of such excesses will be dealt with later.

Important considerations in the interpretation of metabolic balances of the inorganic elements are the observations of Sherman and Gettler (8) and of Bogert and Kirkpatrick (9); the former showed that food, on burning, leaves an ash in which either acid or basic elements may dominate and the latter found, at least with respect to calcium, that acid ash causes a loss, whereas basic ash causes retention. The mode of elimination (urine and feces) appears also to be affected by the reaction of the ash; when the ash of the diet is basic, relatively less calcium is eliminated in the urine than when the ash is acid.

The above observations are referred to, in preference to those noted in animal experiments of a similar nature, for two reasons. Firstly, as they are the result of experiment in man, the data are more comparable. Secondly, the animal data are highly controversial. Thus, though there appears to be general agreement, that calcium-poor diets in the dog, are not conducive to equilibrium

of this element, a statistical investigation of many workers has failed to reveal any definite relationship (10). In spite of apparently carefully planned experiments, diametrically opposite results appear to have been obtained in experiments of a similar and simple type, such as measuring the effects of hydrochloric ingestion (11, 12).

The diet used in this investigation was constructed so as to yield an approximately neutral ash. In order to keep the number of necessary chemical analyses at a minimum, very few food materials were used. The following was the daily diet,—

	grams
Bread	420
Butter	85
Eggs	200
Milk	1680
Orange juice	200

Preliminary calculations of potential acid and basic ash of orange juice, milk and eggs were based upon the data given by Sherman and Gettler. For bread, calculations were based upon the amount of flour, salt, etc., used in the preparation of our hospital bread. The values for flour are given in the above mentioned tables.

In the following table, it will be observed, according to preliminary calculations, that the ash of the diet used was practically neutral; excess acid from the eggs and bread (corresponding to 53 cc. N/1 acid) was practically balanced by excess base from the milk and orange juice (corresponding to about 51 cc. N/1 alkali). Thus,—

Food

Excess ash or base (cc. N/1 solution)

Type	Amount grams	Acid		Base	
		Per 100 grams	Total	Per 100 grams	Total
Eggs	200	11.1	22.2	—	—
Milk	1680	—	—	2.37	39.80
Bread	420	7.36	30.9	—	—
Oranges	200	—	—	5.61	11.22
Total			53.1		51.0

Since the use of food tables is not justified in such experiments for estimation of intake otherwise, it is obvious that they cannot be employed for estimation of ash. As with other analyses, it has been observed repeatedly that actual and assumed values rarely agree. It is also, obviously, not practical to determine reactions of ash before food is used in an experiment, as the analyses are time consuming and the food would, on standing, be unfit for consumption. Preliminary calculations such as the above are, however, of value in order to assist in constructing diets which *approximate* those required. From subsequent analyses of materials saved, the true reaction of the ash may be calculated and the results applied in the interpretation of data.

The method of calculating ash values was identical with that employed by Sherman and Gettler; the amounts of normal acid or alkali, corresponding to the amount of each element used, were calculated firstly. By adding together and balancing the results of all basic and acid forming elements, the daily ash was found to be slightly acid and corresponded to about 26 cc. of a N/1 solution.

TABLE I

DAILY METABOLIC BALANCE. PERIOD 1 (FEB. 6 TO 11)

Day	Water Intake cc.	Water Output cc.	Urine creatinine nitrogen (grams)	Feces (grams)	Na gms.	K gms.	Ca gms.	Mg gms.	Cl gms.	P gms.	S gms.	N gms.	Daily intake ←
					3.39	3.21	2.45	0.28	5.82	2.20	1.39	19.7	
Feb. 6					2.97	3.45	0.46	0.103	5.63	1.61	1.26	17.8	Urine
	3670	3430	0.560	None	—	—	—	—	—	—	—	—	Feces
					2.97	3.45	0.46	0.103	5.63	1.61	1.26	17.8	Total
Feb. 7					3.21	2.87	0.11	0.081	5.21	0.87	1.42	16.4	Urine
	2765	2275	0.586	201	0.29	0.31	1.62	0.066	0.26	0.45	0.21	2.6	Feces
					3.50	3.18	1.73	0.147	5.47	1.32	1.63	19.0	Total
Feb. 8					2.21	3.11	0.33	0.073	6.31	1.11	1.16	17.0	Urine
	2950	2460	0.496	225	0.13	0.11	2.29	0.052	0.17	1.38	0.18	2.9	Feces
					2.34	3.22	2.62	0.125	6.48	2.49	1.34	19.9	Total
Feb. 9					3.56	2.96	0.76	0.064	5.13	0.62	1.43	16.7	Urine
	3050	2610	0.543	150	0.17	0.28	1.35	0.193	0.25	0.34	0.49	2.9	Feces
					3.73	3.24	2.11	0.257	5.38	0.96	1.92	19.6	Total
Feb. 10					2.82	2.67	0.32	0.118	6.89	0.86	1.62	17.7	Urine
	3675	2490	0.560	None	—	—	—	—	—	—	—	—	Feces
					2.82	2.67	0.32	0.118	6.89	0.86	1.62	17.7	Total
Feb. 11					3.96	3.31	0.26	0.042	5.02	0.68	1.61	17.9	Urine
	2370	2850	0.505	201	0.08	0.39	1.29	0.062	0.37	0.49	0.27	2.6	Feces
					4.04	3.70	1.55	0.104	5.39	1.17	1.88	20.6	Total

To avoid monotony, even during such short period experiments, special efforts are made by our Dietitian, Miss Ruth Parke, to vary the forms in which the few articles of food are served. For example, eggs are served as scrambled or in sandwich form, and eggs and milk are combined as egg-nogs, custards, etc.

RESULTS

The results, in detail, of the daily analyses of Period 1 of the experiment are shown in Table I and, briefly, summarized in Table II.

TABLE II

RESUMÉ OF METABOLIC BALANCE. PERIOD 1.
(All quantities in grams.)

	Na	K	Ca	Mg	Cl	P	S	N
Intake	20.34	19.26	14.70	1.680	34.92	13.20	8.34	118.2
Output: Urine	18.73	18.37	2.24	0.481	34.91	5.75	8.50	103.5
Feces	0.67	1.09	6.55	0.373	1.05	2.66	1.15	11.0
Total	19.40	19.46	8.79	0.854	35.96	8.41	9.65	114.5
Balance	−0.94	+0.20	− 5.91	− 0.826	+1.04	− 4.79	+ 1.31	+3.7
Percentage retention or loss	−4.5	+1.0	−40.2	−49.1	+ 2.9	−36.3	+15.7	+3.1
Percentage of total excretion in urine and feces.								
Urine	96.5	94.4	25.0	56.3	97.0	68.3	88.1	90.3
Feces	3.5	5.6	75.0	43.7	3.0	31.7	11.9	9.7

In the discussion of the data, consideration is given to (a) degrees of retention or loss of the different elements, (b) modes of their elimination (relative proportions in urine and feces), (c) possible relationships between amounts of material ingested and excreted and (d) the inter-relationships of the metabolism of the different elements.

Sodium, Potassium and Chlorine

From actual analyses of bone and from other available data, it would appear that the metabolism of sodium, potassium and chlorine differs from other elements to be considered presently; they not only play a small part in the formation of bone, but differ from the other elements also in that the body appears to be able readily to adjust itself to any tendencies towards their deficiencies, though they may not always be interchangeable and, in some of their functions, may actually be antagonistic; the "Law of the Minimum" appears to be able to assert itself readily. The operation of this law is strikingly shown in the experiments of Osborne and Mendel (13), those of Goodall and Joslin (14) and in Benedict's classical fasting

experiment (15). This probably explains the observation in our case of osteitis deformans that the patient was practically in equilibrium with respect to these elements. Their modes of elimination were also normal. Past experiences with such work suggest that such negative and positive balances as were found in this case are of limited significance. From repeated observations they appear to be transient and are apparently related to fluctuations of water content of the body—probably a compensatory mechanism in the regulation of osmotic pressure. They are not regarded as pathological.

Calcium

In the interpretation of the data with respect to calcium, appreciation of the intimate association between the metabolism of this element and bone formation is necessary. According to the table given by Sherman (6) on "The elementary composition of the body," calcium stands fifth in the list and constitutes about 1.5 per cent of the total body weight. It thus constitutes a larger proportion of the body weight than any of the other inorganic elements. The calcium content of body tissues is of interest here; though this element is required for a variety of physiological functions other than bone formation, (blood coagulation, etc.) there is a marked disproportion between its importance and the amount required for these functions; practically 99 per cent of all of the calcium is deposited in bone. About 85 per cent of all of the mineral matter of bone is represented by compounds of calcium (phosphate or carbonate) and the actual calcium content of normal human bone, expressed as calcium oxide, is about 28.8 per cent. McCrudden (16) gives the following percentage composition of normal human bone:

CaO	28.85
MgO	0.14
P_2O_5	19.55
S	0.14

Differing from sodium and chlorine, the defensive mechanism against loss or an insufficient supply of calcium does not appear to be very perfect. This is shown in Benedict's fasting subject; on the last (31st) day of the experiment the amount of calcium excreted was still appreciable, namely, about one-half of that found on the first day; whereas, the excretions of sodium and chlorine were practically negligible. Thus:

	Urinary Excretion (gms.)		
Day	Na	Cl	Ca
1st	2.070	3.77	0.217
31st	0.053	0.13	0.138

Table II shows that there was a very marked retention of calcium in our case and the data agree essentially with those recorded by Da Costa *et al.* (1). In the two cases reported by the latter authors, the percentage retentions were 50.3 and 18.0 respectively.[4] As no two of these three patients were on the same diet, this must be considered in the interpretation. As our results are expressed in terms of the element alone, whereas those of the above mentioned authors are expressed in terms of calcium oxide, our data were re-calculated in terms of the oxide in order to make the findings comparable. The following table shows there was no relationship between intake and retention. Thus:

	Calcium (CaO)	
Subject	Intake per day (grams)	Retention (per cent)
W.	2.585	18.0
B. (our case)	3.430	40.2
McD.	1.598	50.3

Normally, though restricted intake may lead to loss of body calcium (6), excess intake appears to have relatively little effect upon retention. In one of Sherman's experiments it was found that when the calcium content of the food was increased 250 per cent above the normal, 13 per cent only of the ingested calcium was retained (17). As the ingestions of calcium by the above mentioned subjects were not anywhere near those used in Sherman's experiment, some other explanation must be sought for the values found.

The relative proportions of calcium found in urine and feces are of interest. Normally, about 10 to 40 per cent of calcium is found in the urine, whereas the balance—by far the greater portion—is excreted by the intestinal tract (18). Food intake is an influencing factor; when the diet is rich in calcium, a large part of the latter is excreted by the intestines; whereas during fasting, or in conditions comparable to fasting (destruction of bone tissue, etc.) urinary calcium is increased. In one of Sherman's experiments when the calcium intake was very low, a large percentage of this element (42 per cent) was excreted in the urine. In Benedict's fasting man, during the period when no feces were passed, there was an appreciable excretion of urinary calcium. In a case of osteomalacia (19) about 68 per cent of the calcium excreted was found in the urine. A characteristic feature of this condition is destruction of bone with loss of calcium.

[4] Throughout the discussion of this paper these two cases will be referred to frequently as the method of their investigation was identical with that of our own case (nursing procedures, clinically, X-Rays and chemical methods). They will henceforth be designated as subjects W. and McD. respectively. Our case will be designated as subject B.

McCrudden (16) gives the following percentage composition of bone in this disease:

CaO	15.44
MgO	0.57
P_2O_5	12.01
S	0.55

Compared with the analyses given above for normal bone, the calcium content of bone in this disease was about one-half the normal.

The metabolic balance of a case of hyperparathyroidism—another condition which results in bone destruction—studied in this hospital (see Table V), showed that 73.4 per cent of the calcium was found in the urine.

In subjects W. and McD., the urinary calcium excretions, expressed as percentages of total excretions, were 4.6 and 1.0 respectively. In subject B. (our case) (Table II) about 25 per cent of calcium was excreted by the urine. Opposed to these findings are those made by Bruere for Gruner, Scrimger and Foster (20); in this case the urinary excretion was greater than the intake. However, in view of the fact that this case of osteitis deformans was complicated by multiple sarcoma formation and as the latter condition also causes loss of bone tissue, this case may be regarded as at least somewhat comparable to fasting during which body calcium is mobilized and relatively largely excreted by the kidneys.

How are these data to be interpreted? On the basis of X-Ray findings, Da Costa *et al.* (1) stressed calcification of arteries and of the pineal gland as a possible explanation of part of the calcium retention. In subject B., at least, the opinion is that the calcium retained was deposited chiefly in the bones; the X-Rays showed no calcification of arteries, and, with regard to the pineal gland, it may here be observed, as our Roentgenologist, Dr. W. L. Ritchie pointed out, that calcification is now regarded as a fairly common phenomenon; the incidence is about 30 per cent of all examinations made in this hospital to date. As a matter of fact, the condition is so common as to warrant little comment. It is of interest, however, to note, from actual chemical analysis, that though an increase of calcium in brain tissue was found to be uncommon, the calcium content was high in a case of Paget's disease (21).

The degree of retention of calcium in subject B. (our case) would suggest calcium starvation; the proportion of the total excretion found in the urine also corresponds to that found in starvation or destruction of bone, when the intake is normal. Since, as stated above, practically 99 per cent of calcium is found in bone, the most probable explanation is that, in osteitis deformans, there is simultaneously destruction of bone with loss of calcium

and an attempt at compensatory calcification. Available data are, however, conflicting. In subjects W. and McD., the X-Rays indicated calcification of the newly formed bone, but in subject B. (our case) there was no such evidence. An investigation of past records in this hospital, however, showed excess calcification in the majority of cases. Chemical analyses of bone also favor calcification; in all of the studies the calcium content was increased. Opposed to chemical analyses are histological data (22); these suggest little or no calcification. From an evaluation of the different methods of study (X-Ray, histological and chemical) it is, however, hardly necessary to state that chemical analyses afford the most reliable evidence. In other words, it may definitely be stated that excessive calcification is a characteristic of osteitis deformans.

The above incompatibilities become more apparent than real when due consideration is given to the bone pathology of the disease. As Adami and Nicholls (23) have pointed out, there are two opposing pathological processes, namely, (a) absorption of tissues and (b) osseous hyperplasia. With absorption of tissues there is bone destruction and replacement of bone by a fatty gelatinous or fibrous tissue, poor in cells; with osseous hyperplasia, there is proliferation and new bone formation in the periosteum and bone marrow which leads to increased mass and density. *Eventually* calcification occurs. The latter apparently represents the terminal (healing) process of this disease. As histological changes, X-Ray findings and the result of chemical analyses depend not only upon the degree but the extent and stage of the diseased process, it appears that all of the above data may readily be explained; in no two cases were the conditions comparable. Subject W. was apparently the mildest case and subject B. (our case) may be regarded as intermediate between subjects W. and McD.

It is obviously impossible to determine, with exactness, the severity of the disease. For practical purposes, however, it appears reasonable to assume that when the X-Rays show "rarefaction" only, the disease is more marked than when "striation" (osteoid formation) is noted; that the presence of subperiosteal deposits with increased diameter of bone indicates a less active lesion than the latter; and evidence of calcification suggests a healing, and therefore less severe, lesion. If these qualitative changes are correlated with the *number* of bones involved and the *extent* of the lesion in each bone, a classification with respect to severity appears possible. On this basis, the severity of the disease in the cases under discussion is, as stated above, as follows:

1.—W. 2.—B. (our case) 3.—McD.

Thus: In subject W., X-Rays not only showed calcification but involvement of two bones only, namely, the right tibia and upper end of the right femur; in subject B. (our case) there was no calcification, but more bones were involved; the lesions included both femora, the right humerus, the right tibia and the skull bones; whereas, in subject McD., though there was calcification, the disease was very widespread and included both clavicles, both scapulae, both humeri, both radii, both ulnae, the third metacarpal of the right hand, the index metacarpal of the left hand, many ribs, all of the lumbar vertebrae, all the pelvic bones, both femora, right patella, both tibiae, both fibulae, the astragalus and the os calcis on the right side. It is of interest to note that calcium retention and severity, according to the above method of classification, were parallel; the percentage retentions of calcium in the three cases were 18, 40.2 and 50.3 respectively.

The X-Ray finding of calcification in subject W., with a calcium retention of 18 per cent only and the absence of this change in subject B. with a 40.2 per cent retention of calcium may, at first, appear incompatible. An explanation suggested is that, though subject B. (our case) showed much more marked retention, the calcium was still, because of the more severe lesion, in some organic form and permeable to the X-Rays. Of interest with regard to the interpretation of this phenomenon are the observations of Taylor and Sheard as a result of their microscopic and X-Ray studies of calcification of tissues (24).

From the above it appears reasonable, though no observations have been recorded hitherto, to conclude that a metabolic balance obtained in the very early stage of the disease would probably show excess calcium excretion (i.e., loss) rather than retention; and a large proportion of this element would be eliminated in the urine. This may appear incompatible with the data in these three cases; the percentages of urinary excretion of calcium did not parallel severity; for subjects W., B. and McD. they were 4.6, 25 and 1.0 respectively. However, in no two of these subjects was the calcium content of the diet the same. Thus:

CALCIUM OXIDE

Subject	Intake (gms.)	Output in urine (% of total)
W.	2.585	4.6
B. (our case)	3.430	25.0
McD.	1.598	1.0

This relationship between intake and excretion will again be referred to in the general discussion of the results.

Magnesium

Interpretation of magnesium data is much more difficult than those of calcium, since very little is known of the physiological functions of this element. This probably explains the meagre consideration magnesium has been given clinically. That which is known, and relevant here, may be briefly summarized.

That magnesium is intimately concerned with the metabolism of bone is suggested from the fact that about two-thirds of the total amount in the body is found in this tissue (25). It exists in bone as the solid phosphate and carbonate. According to the above analysis, given by McCrudden, it forms a very small fragment of the solid inorganic matter of bone. Differing from calcium, magnesium appears to be very widely distributed in nature and the body appears able to adapt itself readily, as in the case of potassium, sodium and chlorine, to tendency towards deficiencies. With regard to its elimination, again differing from calcium, the kidneys and intestines play an approximately equal part, under normal conditions (18). There, however, appears to be an intimate relationship between calcium and magnesium metabolism, at least with respect to bone. Bogert and McKittrick (26) have shown that excessive intake of calcium may lead to excessive excretion of magnesium and vice versa. In spite of this antagonism, deposition of magnesium in bone is presumably determined by the same factors which account for deposition of calcium (27). This would suggest itself from the closely allied chemical nature of these two elements, according to their position in the Mendeljeff table. This probably also explains the observation that one element can replace the other. For example, in osteomalacia (19), loss of calcium was accompanied by retention of magnesium and the same phenomenon was observed in our case of hyperparathyroidism (Table V). The metabolic balance in the latter case showed a 50 per cent retention of magnesium. There is, also, indirect evidence (28, 29) that, though calcium and magnesium may not ordinarily subserve the same function with the organism, they can, apparently, to a certain extent at least, replace one another. It is of interest, here, to note that another element in the same column of the table of elements as magnesium has been found able to substitute for calcium, namely, strontium (30). This phenomenon will again be referred to in the interpretation of the data.

The data in subject B. are interpreted on a basis of the above observations. On a daily intake of 2.772 grams of magnesium oxide there was marked retention, namely 49.1 per cent. Again, if the three subjects are arranged in order of severity, there appears to be some relationship between the latter and degree of retention. Thus:

Subject	Daily intake (gms.)	Percentage retention
W.	4.229	35.1
B. (our case)	2.772	49.1
McD.	2.768	58.7

As the greater part of the magnesium is in bone, as the factors which govern deposition of calcium also govern that of magnesium, and as magnesium may substitute for calcium when the latter is in great demand, interpretation of the above findings does not appear difficult.

There appears to be no abnormality with respect to the mode of elimination; slightly over one-half of the total amount of magnesium eliminated was excreted by the kidneys.

Phosphorus

The intimate relationship between the metabolism of phosphorus and bone formation is obvious. Phosphorus is the limiting factor in bone formation. About 20 per cent of bone is composed of phosphorus, calculated as P_2O_5. It and calcium are the essential constituents of the rigid supporting structure of all osseous tissue. It has been calculated that the body contains about 700 grams of phosphorus and 600 of the latter is found in bone. Though phosphorus may exist in many forms (nucleo-proteins, phosphatides, in combination with starch, etc.), it appears, from available data, that it is permissible to compute the total phosphorus intake in a metabolic balance experiment without regard to separate computation with respect to the different forms and different sources of this element. From the point of view of bone pathology, it is of interest to note that, as in the case of calcium, compared with chlorine, sodium and potassium, the body is poorly fitted to cope with tendencies toward deficiencies; liberal amounts of phosphorus are always required to maintain equilibrium.

As with calcium and magnesium, there was a marked retention of phosphorus in subject B. On an intake of 5.060 grams, calculated as P_2O_5, there was a retention of 36.3 per cent. Again, there appears to be some relationship between the severity of the disease and degree of retention. Thus:

Subject	Daily intake (gms.)	Percentage retention
W.	4.300	28.6
B. (our case)	5.060	36.3
McD.	3.972	50.3

As the excretion of phosphorus is greatly influenced by the rate of metabolism and intake (6), these factors are excluded in subject B. at least; the intake was not only sufficient under ordinary conditions, but slightly in excess; the basal metabolic rate was also normal (+6 per cent).

With regard to the mode of elimination, here, there was nothing abnormal to note; about 65 per cent was excreted by the kidneys and the remainder by the intestines.

Sulfur

The sulfur metabolism is of particular interest in that, differing from the other elements, the metabolic balance showed a definite loss (Table II) and, as with the other elements, there appeared to be some relationship between severity of the disease and excretion. Thus:

Subject	Daily intake (gms.)	Percentage
W.	0.769	2.3 retention
B. (our case)	1.39	15.7 loss
McD.	0.612	43.8 loss

The sulfur metabolism data are as difficult to interpret as those of magnesium, also because of our limited knowledge of the physiology of this element. Analysis of bone (McCrudden) shows that it contains very little of this element. It is very difficult to attribute loss of sulfur to destruction of bone alone, since, in osteomalacia, sulfur was found to be retained rather than lost (16,19) and in our case of hyper parathyroidism (Table V) there was also a retention of sulfur.

Difficult to reconcile are the nitrogen and sulfur data. Under ordinary conditions, the amount of urinary sulfur excreted tends to parallel protein catabolism. This is the basis of the use of the so-called N:S ratio. This ratio is, however, not so constant as was originally supposed; it has been found to vary between 5:1 to 13:1 or more, and is affected by a variety of conditions. Thus, in fasting, the value is lower in the early stages than in the late period, suggesting, as Peters and Van Slyke point out (27), that nitrogen in excess of sulfur is preferentially being retained in the body. This attempt, during fasting or conditions comparable to fasting with respect to loss of body tissue, to retain nitrogen is also sug-

gested from the following data in the three cases of osteitis deformans. Thus:

	Percentage Retention or Loss	
Subject	Sulfur	Nitrogen
W.	2.3 retention	1.0 retention
B.	15.7 loss	3.1 loss
McD.	43.8 loss	1.06 loss

Interrelationship of Elements

A number of workers have calculated ratios of one element to another in an attempt to interpret the data obtained in metabolic balance experiments in osteitis deformans. For example, the ratio of calcium to magnesium has been found similar to that of growing bone (1) and, on the basis of observed ratios of nitrogen to sulfur, attempts have been made to differentiate the character of the organic matrix laid down in diseased bone from that of normal bone (19). Our ratios, when calculated, do not agree with the different reports and an explanation of the differences is suggested in the following observations:

The type of metabolic balance experiment indicated in a given case depends upon the information sought. For example, as Sherman points out (6), "maintenance requirement" experiments differ from those when studying "requirements during growth" or disease associated with abnormal demands of the different elements. With respect to the element under consideration, in order to determine maintenance requirements, the intake and output are measured on diets of normal character, but low in that element, until one finds the minimum amount which will just permit the maintenance of equilibrium. On the other hand, in studying the phenomenon of growth, the plan is to find the intake which will support an optimum rate of storage of the element in question in the growing body. As far as we have been able to ascertain, experiments corresponding to those required to interpret ratios of one element to another have not been performed in cases of osteitis deformans; all have been of the same type as our own. In view of this fact and our lack of information with respect to the selective properties of bone in this disease when exposed to diets containing different mixtures of the different elements, it is obvious that limited significance must be attached to the ratios recorded.

An additional difficulty with regard to the interpretation of such ratios as calcium to phosphorus and magnesium to phosphorus is the fact that bone is not absolutely constant in its inorganic composition; both ratios of one element to another and one compound to another have been found

TABLE III

DAILY METABOLIC BALANCE. PERIOD 2 (MAR. 9 TO 14)

Day	Water Intake cc.	Water Output cc.	Urine creatinine nitrogen (grams)	Feces (grams)	Na gms. 3.61	K gms. 3.28	Ca gms. 2.25	Mg gms. 0.29	Cl gms. 5.92	P gms. 2.10	S gms. 1.38	N gms. 21.7	Daily intake
Mar. 9					3.33	2.65	0.21	0.087	5.36	0.92	1.46	20.6	Urine
	2470	2160	0.513	None	—	—	—	—	—	—	—	—	Feces
					3.33	2.65	0.21	0.087	5.36	0.92	1.46	20.6	Total
Mar. 10					3.11	3.16	0.63	0.062	5.29	0.75	1.68	19.9	Urine
	2960	2680	0.486	130	0.26	0.22	1.03	0.074	0.37	0.46	0.22	1.0	Feces
					3.37	3.38	1.66	0.136	5.66	1.21	1.90	20.9	Total
Mar. 11					4.21	3.46	0.29	0.053	6.13	1.34	1.21	20.0	Urine
	3085	2650	0.453	103	0.26	0.11	0.94	0.096	0.21	0.63	0.13	2.1	Feces
					4.47	3.57	1.23	0.149	6.34	1.97	1.34	22.1	Total
Mar. 12					3.86	3.33	0.32	0.046	5.87	2.24	1.63	19.3	Urine
	2560	2460	0.534	100	0.11	0.06	1.26	0.096	0.16	1.18	0.23	2.2	Feces
					3.97	3.39	1.58	0.142	6.03	3.42	1.86	21.5	Total
Mar. 13					3.65	2.68	0.16	0.098	5.93	0.63	1.67	19.8	Urine
	2500	2650	0.503	35	0.07	0.11	1.97	0.141	0.12	0.55	0.18	1.6	Feces
					3.72	2.79	2.13	0.239	6.05	1.18	1.85	21.4	Total
Mar. 14					3.10	3.21	0.09	0.092	5.64	0.92	1.29	20.3	Urine
	2850	2480	0.469	110	0.12	0.47	0.87	0.106	0.38	0.83	0.16	1.5	Feces
					3.22	3.68	0.96	0.198	6.02	1.75	1.45	21.8	Total

to vary widely. The picture is complicated still further by recent work which suggests that calcium and phosphorus may be deposited in bone, not as $(Ca)_3 (PO_4)_2$ but as Ca H PO_4. It has been claimed that solubility products in blood and the composition of bone agree better with this hypothesis than with the generally accepted idea. The microscopic and X-Ray investigation of calcification of tissue by Taylor and Sheard are, however, of particular interest here; X-Rays and refractive methods have revealed no secondary phosphate in bone.

A metabolic balance was, again, obtained about one month later; the phenomena observed were essentially the same. The details of this experiment are shown in Table III and, briefly, summarized in Table IV. During the interval between the two experiments, the patient received an intensive course of quartz light treatment. The latter, obviously, was without effect, at least with respect to the inorganic elements. This is of interest with regard to vitamin deficiency as a possible cause of this disease. This possibility will be referred to under the discussion of "etiology."

TABLE IV

RESUMÉ OF METABOLIC BALANCE. PERIOD 2.

(All quantities in grams.)

	Na	K	Ca	Mg	Cl	P	S	N
Intake	21.66	19.68	13.50	1.740	35.52	12.60	8.28	130.3
Output: Urine	21.26	18.49	1.70	0.438	34.22	6.80	8.94	119.9
Feces	0.82	0.97	6.07	0.513	1.24	3.65	0.92	8.4
Total	22.08	19.46	7.77	0.951	35.46	10.45	9.86	128.3
Balance	+0.42	−0.22	−5.73	−0.789	−0.06	−2.15	+1.58	−2.0
Percentage retention or loss	1.8	1.1	42.4	45.3	0.2	17.0	19.0	1.3
Percentage of total excretion in urine and feces.								
Urine	96.2	94.8	21.8	46.0	96.2	65.0	90.6	93.4
Feces	3.8	5.2	78.2	54.0	3.8	35.0	9.4	6.6

Etiology

In their discussion on etiology, Da Costa *et al.* (1) suggest a number of possibilities; and these include disturbances of the ductless glands. Since their report in 1915, however, material advance has been made in the study of internal secretions (parathyroid, thyroid, pancreas; adrenals, pituitary, etc.) and, though our knowledge is by no means complete, it appears possible to exclude a number of conditions. Material advancement

has also been made with respect to vitamins and the conditions which govern acid-basic equilibrium, and, as is well known, both of the latter influence the metabolism of the inorganic elements.

There appears to be little doubt that inorganic metabolism can be influenced by the thyroid gland; thyroid feeding and overactivity (hyperthyroidism) cause increased excretion of calcium and phosphorus; whereas hypothyroidism has a diametrically opposite effect (31). However, the sulfur metabolism in the above conditions appears to differ fundamentally from that in osteitis deformans; it, apparently, bears little or no relationship to the metabolism of the other inorganic elements; sulfur excretion appears to be dependent upon protein metabolism alone; excretions of sulfur and nitrogen are parallel and the N:S ratio is constant. This probably explains the finding of a constant ratio of N:S in spite of excess calcium excretion after nitrogen equilibrium was established (32). Hyperthyroidism and hypothyroidism are also excluded on the basis of the clinical condition and basal metabolic rate data. Boothby and Sandiford (33) found the basal metabolic rate normal in five of six cases of osteitis deformans and in our case (subject B) it was also normal, namely +6 per cent. The blood sugar time curve also tends to exclude hyperthyroidism.

TABLE V
RESUMÉ OF METABOLIC BALANCE IN A CASE OF HYPERPARATHYROIDISM
(Period of observation 10 days.)
(All quantities in grams.)

	Na	K	Ca	Mg	Cl	P	S	N
Intake	34.81	35.63	21.67	2.54	58.50	21.40	12.40	197.0
Output: Urine	30.30	30.67	20.04	0.77	57.34	18.48	9.15	174.0
Feces	2.18	2.74	7.23	0.44	2.71	9.52	1.52	13.5
Total	32.48	33.41	27.27	1.21	60.05	28.00	10.67	187.5
Balance	−2.33	−2.22	+5.60	−1.33	+1.55	+6.60	−1.73	−9.5
Percentage of total excretion in urine and feces.								
Urine	93.2	91.8	73.4	63.6	95.4	66.0	85.7	92.8
Feces	6.8	8.2	26.6	36.4	4.6	34.0	14.3	7.2

Though, as stated above, it is quite possible, in the early stages of osteitis deformans, that the disease is associated with loss of calcium, and thus agrees with hyperparathyroidism, other features, particularly the metabolic balance shown in Table V, appear to exclude excessive activity of

the parathyroids as a cause; from the stage of the disease and number of bones involved, the lesion in our case of hyperparathyroidism differs little with respect to its severity (according to the above mentioned classification of severity) from the case of osteitis deformans. The results of the metabolic balances differed, however, very greatly; in the former case there was loss of calcium and retention of sulfur; whereas in the latter there was retention of calcium and loss of sulfur. Incidentally, the serum calcium was markedly increased in the former case, namely, 14.2 mgms. per 100 cc., whereas in the case of osteitis deformans it was normal (9.2 mgms. per 100 cc.). Koechig (34) reports high calcium values obtained in two cases, namely, 12.1 and 11.8 mgms. per 100 cc. However, Lyman's method (35) and citrated blood were used. It may here be observed that plasma tends to yield higher values than serum.

With regard to the known internal secretion of the pancreas (insulin) our records show two cases only of Paget's disease, amongst over 3000 patients in the diabetic clinic of this hospital. On a statistical basis, therefore, this is excluded as a possible cause.

The clinical picture (complaints, pulse, blood pressure, skin, etc.) and laboratory data (B.M.R., sugar tolerance, etc.) exclude, at least obvious hypo- and hyper-adrenalinism.

The practically neutral diet, pH of blood, CO_2 combining power of the plasma and urine acidity, exclude disturbance of acid-basic equilibrium as a cause.

Vitamins were considered as a possible etiological factor. That vitamins may affect the metabolism of bone is too well known to require further comment. The intimate relationship between vitamin D and calcium metabolism is an example. However, at least in our case, the environment of the patient and his dietary habits appear to exclude osteitis deformans from the group of vitamin deficiency diseases.

Abnormal enzyme (phosphatase) activity was considered as a possible factor. The observations of Kay are of interest here (36). The enzyme phosphatase has apparently the property of hydrolysing soluble calcium salts of phosphoric esters to insoluble calcium phosphate. It is present in bone in localities in which deposits of calcium phosphate are proceeding most rapidly. Excessive plasma phosphatase appears to be confined to bone disease and the highest values were found in osteitis deformans. Phosphatase has no diagnostic significance; it does not differentiate the forms of bone lesions. The conclusion, however, to be drawn from available data to date is that the high values found in bone disease are probably the result rather than the cause of the bone lesions.

The conclusion to be drawn from the above observations is that the etiology of osteitis deformans is, as yet, unknown. The probability, however, is that it belongs to the "metabolic" diseases rather than due to infection. Interpretation of the inorganic balance is difficult; calcium, magnesium and phosphorus data appear to depend upon the stage, severity and extent of the disease and similar findings may be met with in other bone lesions. The sulfur metabolism, however, appears to be a differentiating factor, being confined exclusively to osteitis deformans.

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THE ADDITION OF RAW BEEF OR MEAT SCRAP TO A WHEAT-MILK DIET*

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In May, 1926, a group of albino rats was obtained from Wistar Institute, Philadelphia, and placed upon Sherman Diet B (1) which consists of 2/3 ground whole wheat, 1/3 dried whole milk, with the addition of sodium chloride to the extent of 2 per cent of the weight of the wheat. Although they continued to grow and young were born, the number per litter was small and the weight of those raised to the weaning age, 28 days, was less than that desirable for nutritional studies. Of 11 pregnant females, out of 12 mated, 3 ate their litters and the remaining 8 produced 53 young. Of these, 45 were weaned at 4 weeks of age and placed upon Diet B, but 27 of them died during the 2 weeks immediately after weaning. The condition of those that died was characterized by a sluggishness, failure to eat and a wetting about the pubic area.

The fact that the dead or dying young were eaten by their litter mates suggested the feeding of raw meat and workers in other laboratories informed us that raw meat sometimes brought about improvement in the condition of a breeding colony. Sherman (2) in 1925 mentioned the feeding of raw beef to mother rats as a supplement to Diet B and later Mac Leod (3) reported improvement in reproduction when fresh meat or dried meat was added to certain diets. Hence the practice of feeding 5 gm. of raw beef steak per adult rat in the breeding colony, daily, except Sunday, was begun. Two weeks after the beginning of the meat feeding the same 12 females were again mated. Ten litters were produced totalling 89 living young and 2 dead. In some instances the litters were reduced to 7 young but none died of natural causes before the weaning ages, 21 or 28 days. After weaning they were used successfully for nutritional studies and for breeding.

The improvement in the condition of the breeding stock and of the young produced in the preliminary trials with raw beef, led to the regular practice of feeding it, as a supplement to the Sherman Diet B, to the breeding colony and to young animals selected as breeding stock. Individual

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portions were not given to the nursing young but they obtained small amounts from the portion given to the mother. Since by far the greater part of the meat was consumed by the mother it is assumed that the improved condition of the young was due, in part at least, to improved lactation. A summary of the results obtained when the raw beef supplement was fed is given in Table II, Diet BM.

This paper reports our experience with the unsupplemented Sherman Diet B, with this diet supplemented with raw beef, Diet BM, and with a meat scrap incorporated in the diet, Diet BMS.

Colony Management

It is our practice to mate young breeding stock for the first time at 120 days of age and to use them for breeding until 4 litters have been obtained. This is usually accomplished in a period of 10 months, at least a 2-week's rest period being allowed between weaning and the next mating. The mating ratio has been 1 male to 2 females. Only males weighing 290 gm. to 300 gm. or more, and females weighing 190 gm. to 200 gm. or more at 120 days are used for the breeding colony.

During the first week of life, usually within the first 3 days, the number of young in a litter is reduced to 7. If a female in good condition is available with a litter of the same age as that to be reduced, but less than 7 in number, young are given to her to nurse. The reduction to 7 in a litter was not practiced when the colony was first started but it has been the custom during the greater part of the time covered by this report.

If a female fails to become pregnant after being with a male for 4 weeks, she is placed with another male for the same length of time. If after a third mating she does not become pregnant she is considered sterile and is discarded. Each placing with a male, whether it resulted in a pregnancy or not, is counted as a mating.

Experiment 1

In order to find out whether some other supplement could be used in place of raw beef, preliminary feeding trials were made with small groups of animals in which meat scrap[1] and dried yeast were compared with raw beef as supplements to Sherman Diet B. A group receiving unsupplemented

[1] According to the Purdue Univ. Agric. Expt. Sta. Circ., No. 147 (1927), "Meat scrap and meat meal are ground residues from animal tissues, exclusive of hoof and horn, and contain less than 10 per cent phosphoric acid (P_2O_5)." Swift's meat scrap, 55 per cent protein, was used in the present study.

Diet B was also used. The animals employed were born and reared to weaning at 28 days on Sherman Diet B supplemented with raw beef. Immediately upon weaning they were placed upon the experimental régime. All groups were started at the same season of the year, September, 1927, and mated at 4 months of age.

Each experimental group received Sherman Diet B *ad libitum*. To Group 1 was given a supplement of 5 gm. of fresh raw, lean beef per rat per day. Each individual of Group 2 received at first a daily supplement of 1 gm. and later of 2 gm. of meat scrap until 4 months of age when 10 per cent was incorporated in the diet. Dried yeast, 300 mg. per day, was given to each member of Group 3. A supplement was not given to Group 4.

An attempt was made during a 5-months' period to rear 2 litters to 21 days of age in the case of each female. This was accomplished in the meat and meat scrap groups by the first and second matings, but in the other two groups a larger number of matings was necessary, as indicated in Table I. The average number born per litter in the control group was the smallest of the 4 groups. Furthermore, the average weight of young of the yeast and control groups, at 21 days of age, was definitely less than in the instance of the meat and meat scrap groups, and too low for experimental purposes. At 4 months of age the animals raised for breeding on the meat and meat scrap diets were considerably heavier than those of the other two groups.

Although only small numbers of individuals were employed, the results are quite conclusive that growth of breeding stock to the mating age, reproduction and growth of young are markedly better when raw beef or meat scrap is used with Diet B. The meat scrap is equivalent to raw beef in these respects and both are superior to dry yeast. Sherman and Campbell (1) note that Diet B "is probably capable of further improvement."

Experiment 2

On the basis of the results of Experiment 1, 10 per cent of meat scrap was incorporated in Sherman Diet B, at the proportionate expense of the wheat and milk, for part of the breeding colony. The confirmation of the preliminary trials led to the discontinuance of the feeding of fresh meat and the exclusive use of Diet BMS. In practice the diet used in the colony, Diet BMS, consists of 60 per cent ground whole wheat, 30 per cent dried whole milk[2] and 10 per cent meat scrap (Swift's) with the addition of sodium chloride to the extent of 2 per cent of the weight of the wheat.

[2] Klim.

TABLE I
RESULTS OF PRELIMINARY SUPPLEMENTATION TRIALS

No. of animals	Sex	Av. weight at 28 days†	Av. weight at 4 mos.	No. of matings	No. of pregnancies	No. of litters	No. of young born	Av. no. born per litter	No. alive at 21 days*	Av. no. per litter at 21 days*	Av. weight at 21 days
		gm.	gm.								gm.
Group 1, Sherman B+raw beef (Diet BM)											
4	M	68	361								
6	F	64	225	12	12	12	122	10.1	77	6.4	43
Group 2, Sherman B+meat scrap (Diet BMS)											
4	M	63	324								
5	F	65	211	10	10	10	108	10.8	69	6.9	44
Group 3, Sherman B+yeast (Diet BY)											
5	M	65	271								
4	F	63	188	9	6	6	68	11.3	42	7.0	32
Group 4, Sherman B (Diet B)											
5	M	63	278								
5	F	59	188	15	11	10	75	7.5	35	5.9	27

† On Diet BM until weaning.
* Litters were reduced to 7 young during the first week of life.

Although the use of the above modification of the Sherman Diet B has been successful, as demonstrated in Tables II and IV, another attempt was made, beginning in January, 1930, to use the original Sherman Diet B. This trial was made after the breeding colony had been on Diet BMS for 2 years and the young used were born and reared to 28 days on this diet. At weaning, 28 days, 38 female litter mates from 15 litters were divided into groups of 19 each, one of which received Sherman Diet B and the other Diet BMS. The males were selected from the same group of litters so that litter mates appeared in each of the experimental groups. Mating was begun at 120 days or shortly thereafter and was conducted so that each female had an opportunity to have two litters.

TABLE II

SUMMARY OF REPRODUCTION AND GROWTH RECORDS ON DIETS BM, BMS AND B*

	Diet BM	Diet BMS	Diet B
No. of females at 28 days	47	159	26
Av. weight at 28 days	55.6 gm.	62 gm.	57 gm.†
No. of females at 120 days	45	156	26
Av. weight at 120 days	196 gm.	198 gm.	183 gm.
No. of litters observed	108	405	41
Total no. of young observed	903	3600	261
Total no. of young observed alive	869	3361	220
Per cent of total observed alive	96.2	93.5	84.3
Av. no. of young alive per litter at birth	8.0	8.3	5.4
Percentage of young born alive which died during first 7 days of life from natural causes	5.7	6.2	61.9
Nursing at 7 days of age:			
No.‡	702	2476	70
Av. weight	14.0 gm.	14.0 gm.	9.8 gm.
Nursing at 21 days of age:			
No.	656	2437	66
Av. weight	40.5	40.6	25.6

* Includes animals reported in Tables I and III.

† Born and reared to 28 days of age on Diet BM or BMS.

‡ Litters reduced to 7 young during 1st week.

The results are essentially those observed in previous trials and are summarized in Table III. The colony routine of discarding males less than 290 gm. to 300 gm. in weight, and of females of less than 190 gm. to 200 gm. in weight at 120 days, was practiced with regard to the animals on Diet BMS but not in the case of Sherman Diet B because only a few reached this standard. Litters were observed within 18 hours after birth.

TABLE III

EXPERIMENT 2

COMPARISON OF REPRODUCTION AND GROWTH RECORDS, DIETS B AND BMS

	Diet B	Diet BMS
At 28 days of age:		
No. of females	19	19
Av. weight	56.3 gm.†	58.5 gm.
At 120 days of age:		
No. of females used for mating	19	15*
Av. weight	180 gm.	208 gm.
No. of litters observed	25	27
Number born:		
Observed living	139	262
Observed dead	36	2
Average number observed alive per litter	5.5	9.7
Percentage mortality during first seven days due to natural causes	80 per cent	2.4 per cent
No. killed in reducing litters to 7 young	2	80
Nursing at 7 days:		
Number	26	176
Av. weight	9.2 gm.	14.5 gm.
Nursing at 21 days:		
Number	24	176
Av. weight	24 gm.	40.5 gm.

* 4 of the original 19 were killed at 4 mos. because their weights were less than 200 gm. Only 2 of the Diet B group reached this weight at this age.

† Born and reared to 28 days of age on Diet BMS.

The infant mortality of the young observed alive on Diet BMS was 2.4 per cent during the first 7 days, whereas that in the case of Sherman Diet B was 80.0 per cent. However, there was no mortality on the former and only 2 deaths on the latter diet between 7 and 21 days. Reduction of litters to 7 young during the first week of life was practiced in both groups. The young of Diet BMS were considered to be of satisfactory weight for experimental work, averaging 40.6 gm. at 3 weeks, and they were in a healthy, vigorous condition. Those of Diet B were not heavy enough for experimental use and they did not have the healthy appearance of the animals of the meat scrap group.

The Growth of Young on Sherman Diet B After Weaning

During the time that the feeding of raw beef was practiced, the assumption was made that the meat improved lactation and that consequently it might not be necessary to supply it during the growth period

from weaning until the mating age, 120 days. As a test of this assumption 16 males and 22 females, from parents which had received raw beef, were weaned at 28 days (May, 1927) and placed upon Sherman Diet B without the meat supplement. After 11 days on this feeding régime, they began to lose weight and to show nutritional disturbances and, from 11 to 21 days after weaning, 29 died. The condition resembled closely that of the young which were placed on Diet B in July, 1926, when the colony was started. The survivors were given raw beef and an immediate improvement in condition and a resumption of growth took place.

On the other hand the animals used in Experiment 1, started in September, 1927, and those of Experiment 2, started in January, 1930, grew to the mating age on the Sherman Diet B and did not show the nutritional disturbances and cessation of growth noted above. Unless there is a seasonal influence, no reason is apparent as to why the different responses occurred.

The Protein, Calcium and Phosphorus Contents of the Rations

The inclusion of a meat scrap in Diet B caused an increase in the protein content from 19.00 per cent (Diet B) to 22.02 per cent (Diet BMS). Likewise there was an increase in calcium from 0.33 per cent to 1.19 per cent and of phosphorus from 0.49 per cent to 0.89 per cent.

The Ca:P ratio for Diet B used in this laboratory is 0.67 and that for Diet BMS 1.34. Smith and Bing (5) report 0.65 as the Ca:P ratio for Diet B and 1.16 as the ratio when one-half of the sodium chloride is replaced by calcium carbonate.

Discussion of Results

In Tables II and IV reproduction and growth records on Diets BM, BMS, and B are compared. All of the females reported in Table II were not mated the same number of times and therefore the fact that the number of litters per female is smallest in the case of Diet B is not due wholly to poorer reproduction. A detailed study of the colony records show the percentage of fertile females on Diet B to be about the same as on the other diets, yet the pregnancies are fewer when equal mating opportunity is afforded. The mortality of young from natural causes during the first week of life is markedly high in the case of Diet B. The average weights of young at 7 and 21 days on Diets BM and BMS are practically the same, and higher than those of Diet B. In Table II the average weights at these ages were calculated from much larger numbers of Diet BM and Diet BMS animals but in Table III the numbers are nearer the same order, and the same degree of difference in average weight prevails. The small number of

animals available on Diet B for weighing at 7 and 21 days is due to the smaller number observed alive per litter at birth, as compared with the other diets and to the heavy mortality during the first week of life.

The growth of male animals is summarized in Table IV. Those which received the raw beef supplement had been born and reared on this same dietary régime, whereas those on Diet BMS and Sherman Diet B were born and reared to 28 days of age on the former diet. As in the case of the females, Tables II and III, the rate of growth to the mating age on Diet B was less than that on the other two diets.

TABLE IV

GROWTH OF MALES ON DIETS BM, BMS AND B

Age	Diet BM		Diet BMS		Diet B	
	No. of animals	Weight gm.	No. of animals	Weight gm.	No. of aimals	Weight gm.
28 days.....	28	61	78	67.2	24	60.6*
120 days.....	25	292	78	300	17	259
9 mos.....	25	383	69	386	15	328

* Born and reared to 28 days of age on Diet BM or BMS.

It is recognized that animals may not react favorably when changed from one diet to another but the change from Diet BMS to Diet B or from Diet BM to Diet B could scarcely be considered drastic. Certainly such a change would not be so drastic as that from a breeding diet to a vitamin-deficient diet. Furthermore, the change from one diet to another was not always accompanied by an immediate response. In the instance of the young breeding stock, reared on Diet BM and changed to Diet B, the failure did not manifest itself until after 11 days on Diet B. Hence poorer reproduction and growth on Diet B can scarcely be ascribed to a change of diet.

Table V presents a comparison of the growth performance of animals on Diet B as reported by Sherman and Campbell (1) with that of animals on the same diet modified by Macy, Outhouse, Long, and Graham (4), by Smith and Bing (5) and on Diets BM and BMS. Macy and associates use Diet B supplemented with fresh cabbage or lettuce and supply lactating animals with fresh cow's milk *ad libitum*. Smith and Bing replace one-half of the sodium chloride of Diet B with calcium carbonate and feed fresh lettuce daily. Lactating rats are supplied with 9 gm. of dried yeast per week.

TABLE V
COMPARISON OF WEIGHTS OF ANIMALS RECEIVING DIETS BMS AND BM WITH THOSE OF OTHER COLONIES

	Sherman and Campbell (1)		Macy et al. (4)		Smith and Bing (5)				Diet BMS				Diet BM			
Age	Ave. wt., males	Ave. wt., females	Ave. wt.,† males	Ave. wt.,† females	No. of males	Av. wt., males	No. of females	Av. wt., females	No. of males	Av. wt., males	No. of females	Av. wt., females	No. of males	Av. wt., males	No. of females	Av. wt., females
Days	gm.	gm.	gm.	gm.		gm.		gm.		gm.		gm.		gm.		gm.
28	42.6	40.5	55	52	53	63 (27)‡	11	60 (27)	78	67.2	159	62	28	61	47	55.6
120	247.1	182.1	286	192	52	330 (115)	21	219 (115)	78	300	156	198	25	292	45	196
235	—	—	370 (224)	230 (224)	29	448	12	235	60	379	96*	247	25	370	27*	256
300	314.9	222.2	—	—	—	—	—	—	47	399	114*	260	25	396	33*	268

† Values taken from curves.
‡ Numbers in parentheses indicate days of age at which weighing was made
* Non-pregnant. Weights taken between pregnancies.

Each of the above modifications of Diet B has brought about an improvement in growth rate. Furthermore, our records displayed in Table II show a higher average weight for young at 7 and 21 days on Diets BM and BMS as compared with Diet B. At 28 days of age the animals of the colony of Macy and associates are slightly less in weight than those reported by Smith and Bing and those of this colony, but with the exception of the weights of females on Diets BM and BMS at 235 days their weights agree closely with those of our colony, and are lower than those reported by Smith and Bing. At 235 days, the females on Diets BM and BMS are slightly heavier than the others listed in Table V.

The reason for the improvement in growth which resulted from the several modifications of Diet B is not known. Smith and Bing state, "It is highly probable that the improved growth observed in our colony is due in large part to the more favorable relationship between calcium and phosphorus as well as to the alteration in the potential reaction of the diet." This tentative conclusion would not be supported by the results obtained in this colony when Diet B is supplemented with raw beef. In this instance there was no addition of calcium, the Ca:P ratio would probably be less than that of Diet B and the potential reaction of the diet would tend to become more acid. Diet B as modified by Smith and Bing became potentially more alkaline. The use of leaf tissue and of fresh milk with Diet B, as practiced by Macy and associates, would tend to increase slightly the Ca:P ratio and the calcium intake, but not as much as the addition of calcium carbonate in the amount used by Smith and Bing.

On the basis of the experience with this colony it would appear that raw beef and meat scrap have some property, not possessed by dry yeast, which improves the growth obtained with Diet B. Although Diet BMS contains 3.6 times as much calcium as Diet B, the improvement cannot be due chiefly to an increase in this element because raw beef causes essentially the same response. The greater growth rate obtained by Macy and associates, by Smith and Bing, and in this colony, may be due largely to a factor common to lettuce, cabbage, meat scrap, and raw beef. An investigation of the cause of the improvement in growth rate has been planned.

Summary

1. The addition of fresh beef or of a meat scrap to a wheat-milk diet, used as a breeding colony ration for white rats, improved reproduction, and the growth rate and general vigor of the young as compared with the performance on the wheat-milk diet.

2. The use of dried yeast with the wheat-milk diet caused a slight improvement in reproduction but the growth rate and general vigor of the young were not bettered.

3. The improved performance on the wheat-milk diet supplemented with fresh beef or with meat scrap cannot be due chiefly to an increase in calcium and phosphorus or to an increase in the Ca:P ratio.

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THE EFFECT OF RACHITOGENIC DIETS ON THE THYROID GLAND OF THE ALBINO RAT

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PATHOLOGICAL changes associated with the feeding of rachitogenic diets have been described in the thyroid gland of dogs and rats. In 1921 E. and M. Mellanby (18) observed hypertrophy and hyperplasia in the thyroids of rachitic dogs. In the following year Murray (19) confirmed their observations, and, furthermore, pointed out that the degree of departure from the normal varied in proportion to the severity of the rickets. Although Davies (3) has not emphasized the fact, her findings, nevertheless, might be considered to cast doubt upon the latter conclusion. From her observations it would appear that greater variation in the thyroid gland occurred with the milder grades of rickets in puppies than with the marked. Sorour (22) noted in the thyroid of rats kept in darkness, and which had developed osteoporosis, a hyperplasia resembling Basedow's disease. In contrast to his results are those of McCarrison (12) who failed to produce goitre in rats kept in darkness fed on a deficient fat-soluble vitamin diet. Recently Krause and Munroe (7) have reported enlargement of the thyroid gland in rats on the Steenbock rachitogenic diet. The various authors have either incidentally mentioned the changes in the thyroid gland or have attributed them to some factor concomitant with the rachitic state. The difficulty of comparing the results obtained by the feeding of a variety of diets, the relatively few animals from which conclusions have been drawn, and the lack of unanimity of opinion, leads one to believe that relatively little is known concerning the relationship existing between these two conditions. Therefore, the present study was undertaken in the hope of demonstrating the factor involved in producing thyroid changes in animals fed on rachitogenic diets.

Experimental

The opportunity to examine a large number of rachitic and non-rachitic rats kept under adequately controlled conditions was afforded by the extensive dietary studies carried on in the Nutritional Research Laboratories of the Sub-Department of Paediatrics, University of Toronto.

Since Sazaki (21), Burget (2), and McCarrison (13–14) have drawn attention to the frequency with which goitre arises in rats kept in an unhygienic environment, all the animals were confined in roomy wire cages with mesh bottoms. They were frequently washed and sterilized. No bedding was used. Usually fewer than five animals were placed in a cage.

The Steenbock rachitogenic diet, consisting of 76 parts of yellow corn, 20 parts wheat gluten, 1 part sodium chloride c.p., and 3 parts calcium carbonate c.p., and City of Toronto water, was employed either alone or with partial substitutions or additions of various ingredients. The experiments may be classified into three general groups, rachitogenic, antirachitogenic, and rachitogenic followed by antirachitogenic or the "line test." The animals were placed on the experimental diets at one month of age for approximately four weeks. The substances used in replacing in part yellow corn were non-irradiated whole wheat biscuit cereal,[1] the same cereal irradiated, plain bread, whole wheat, rolled oats, whole wheat or rolled oats ashed, chocolate, ashed chocolate, egg yolk or egg yolk ashed. Additions to the diets consisted of lard, shortening, viosterol or cod liver oil.

Aside from the routine experiments, a number of special tests were devised. To exclude the possibility of a vitamin deficiency other than D existing, the Steenbock rachitogenic diet was modified. Fourteen parts of wheat gluten and six parts of wheat germ were used in place of twenty parts of wheat gluten. One cc. of tomato juice per rat per day was added. Since it was considered that an inadequate amount of iodine either in the modified or the unmodified Steenbock diet might be a factor, potassium iodide was added to some. Two amounts were used—.00002 gram and .00128 gram per 100 grams of ration. By decreasing the calcium intake in relation to the phosphorus, even in the absence of the antirachitic vitamin, the condition has been considered to be less favorable for the development of rickets in rats (17). Therefore, in order to ascertain if an approximately direct quantitative variation existed between the changes in the thyroid gland and the rachitic phenomena, small groups of animals were placed on the Steenbock rachitogenic diet either with no added calcium or with ¼, 1, 1½ and 2 per cent, instead of the usual 3 per cent of calcium carbonate. Using the modified and the Steenbock rachitogenic diets either with or without iodine, a preventative series of experiments was carried out by supplying vitamin D in the form of either 1 per cent 1D Viosterol or ½ per cent cod liver oil. As much food and water as the animals would voluntarily consume were given.

[1] "Muffetts"

Control rats were those on the normal laboratory diet, as well as a group of wild rats.

The animals were started on these diets at one month of age and two were examined at weekly intervals beginning at the third week. The entire experimental period extended from December to June.

When the desired experimental period had elapsed the animals were etherized. Blood for phosphorus determination was collected by bleeding from the great vessels of the neck. Immediately following this procedure the thyroid glands together with the trachea and oesophagus were secured from two rats in each experimental group and placed in 10 per cent formalin. A representative number were x-rayed. The calcium content of the bones was later determined. After adequate fixation, paraffin sections were made and stained with hematoxylin and eosin. This routine varied in one instance in which the tissues from a group of thirty-two animals were not obtained until five hours had elapsed after death. During this interval the animals were kept in the refrigerator.

Histology

The thyroid glands were classified according to their histological characteristics. Since transitions of one type into another were commonly encountered, it was often perplexing to arrive at a decision as to the particular group into which the specimen was to be placed. A conservative attitude was rigidly adhered to in all such doubtful instances.

Twelve animals one month old, intended to constitute the control group, were placed on the normal laboratory diet, which consisted of 64.5 per cent whole wheat, 15 per cent crude casein, 10 per cent whole milk powder, 8 per cent creamery butter, 1.5 per cent calcium carbonate, 1 per cent sodium chloride, meat and greens twice weekly, and City of Toronto water. One animal was killed weekly and the thyroid examined. For the first three weeks the glands grossly appeared small, and pale pinkish gray in color. Microscopically, the vesicles were small, regular in contour and fairly uniform. A scanty stroma of connective tissue divided them into irregular lobules. The larger vessels were readily demonstrable, but not dilated. Uniformly-staining, thin, colloid filled the acinar space. The lining epithelial cells were flat, having a small amount of acidophilic cytoplasm and an elongated or rounded, deeply-stained nucleus. From the fourth to the tenth week the glands increased in size and in depth of color. The isthmus became prominent. When studied microscopically a variation in the size and shape of the acini was noted. A few presented small plications. Usually the lobular divisions were incomplete. The vascular spaces showed a varying degree of engorgement. The colloid content was diminished and pale-staining, granular and largely vacuolated. The epithelial cells were increased in size, having acidophilic, granular cytoplasm, and enlarged, rounded, centrally placed, reticular nuclei, frequently surrounded by a clear space. Although these latter findings may merely represent physiological activity, rather than pathological change, nevertheless their inconsistency was considered to render them undesirable as a control group.

The difficulty of obtaining thyroid glands in the so-called resting state, led to the study of a series of 22 wild rats obtained from a stable situated in the centre of the city. These rats necessarily subsisted on a very mixed diet. In the gross, nineteen were found to have enlarged, grayish-red glands, while only three appeared of average size and pale, pinkish gray in color. Microscopically, the latter were made up of rather large vesicles which varied in size and shape. Lobular divisions were not readily discerned. No injection of the vessels was noted. The acini were well

filled by dense, granular colloid. The epithelial lining was of the low cuboidal variety, formed by cells having acidophilic non-granular cytoplasm and small, round, reticular or deeply stained nuclei. The former nineteen revealed acini varying markedly in size and shape and were frequently infolded. The majority had engorged vascular channels. The colloid content was depleted. Only pale-staining, granular, vacuolated remnants, partially filling the vesicular lumina, remained. The lining epithelium was of the tall cuboidal or columnar type, having achromatic, granular, occasionally vacuolated cytoplasm, and round or oval, centrally placed, reticular nuclei. Some of the vesicles were lined by more than a single layer of cells. Desquamation of a few cells into the acinar space was not an uncommon finding. Undifferentiated groups of epithelial cells were abundant. From these observations the conclusion drawn was that the thyroid glands of these wild rats showed changes to a degree not compatible with even the most marked variations of physiological activity, but rather the pathological manifestations of struma parenchymatosa diffusa.

Due to the difficulty in establishing a normal, the terms "non-hyperplastic" and "hyperplastic" were adopted in the classifying of the thyroid changes found in the experimental animals. Macroscopically, the non-hyperplastic glands were small and pale pinkish gray in color. Neither the isthmus nor the vessels were prominent. In contrast, the hyperplastic glands were of variable size but enlarged and were a deep grayish red in color. The isthmus was usually clear cut and the vessels outstanding.

The non-hyperplastic glands appeared to fall into two groups. The first group was composed of small and regular acini. The second group differed from the first in the variability of the size of the vesicles, the larger acini predominating. The acini, of both groups were divided into irregular lobules by a meagre stroma of connective tissue. The colloid content was either dense and bland or transparent, homogeneous or finely granular with vacuoles situated chiefly in the periphery. The epithelium lining the acini, containing the larger amount of colloid, was flat. The cytoplasm of the individual cells was small in amount, acidophilic and non-granular. The nuclei were elongated and intensely stained. On the other hand, the alveoli of the second group were lined by poorly staining cells of the low cuboidal variety, with rounded nuclei which stained less intensely than the elongated type. A clear halo frequently surrounded the nucleus. Small groups of undifferentiated epithelial cells, which probably represented collapsed or developing vesicles, were noted in most of the sections. Usually these appeared as syncytial masses having acidophilic cytoplasm. The vessels were not distended.

The hyperplastic glands were divisible into two major classes—those characterized by a predominance of small acini, and those composed essentially of large vesicles. Just as the non-hyperplastic groups had features in common, so had the hyperplastic groups, in that they presented varying degrees of epithelial hyperplasia, graded as slight, moderate or marked.

In the slightly hyperplastic group were placed glands in which the epithelial cells were somewhat larger than normal, having a greater amount of acidophilic granular cytoplasm, and usually showing a clear zone about the nucleus. The nuclei were enlarged, round or oval, pale-staining and reticular, situated more centrally within the cell. Colloid was present in the majority of the acini, but definitely decreased in amount, as indicated by its pale-staining character. Vacuolization was slight or moderate. A variable number of undifferentiated cell accumulations were noted. The vessels were moderately injected.

Glands presenting a moderate degree of hyperplasia were made up of vesicles which presented considerable variation in size and shape. Usually a few of the acinar walls showed plications. The colloid content was still further depleted, being absent in quite a proportion of the acini. That which remained had what might be termed a "washed out" appearance, in that it was pale-staining, thin, granular and largely vacuolated. The epithelial cells resembled those of the slightly hyperplastic groups, although occasionally they were larger. Not infrequently the lining cells were more than a single layer in thickness, often giving rise to small cellular buds protruding into the lumen. Lobular divisions were not discerned. Vascular injection was always present.

TABLE I
EXPERIMENTAL ANIMALS ON RACHITOGENIC DIETS FOR FOUR WEEKS

Rachitogenic Diets	No of animals	X-Ray	Blood[34] phosphorus mgs/100 cc.	Ash content of bones %	Thyroid changes						
					Non-hyperplastic %	Hyperplastic %	Slight hyperplasia	Moderate hyperplasia	Marked hyperplasia		Degenerative
							Small acini / Large acini	Small acini / Large acini	Small acini / Large acini		
Steenbock (a)	34	marked rickets	1.6	29.0	3	80	41%	30%	9%		17%
							29% / 12%	9% / 21%	9% / 0%		
(b), (g), (h)	52	marked rickets	2.1	36.4	0	100	49%	42%	9%		
							32% / 17%	15% / 27%	6% / 3%		
(c), (e)	4	marked rickets	1.4	29.9	100	0					
(d), (f)	6	marked rickets	1.7	32.3	100	0					

The following rachitogenic diets were used:

(a) The unmodified Steenbock rachitogenic diet consisting of 76 per cent yellow corn, 20 per cent wheat gluten, 1 per cent sodium chloride c.p., 3 per cent calcium carbonate c.p.

(b) The Steenbock rachitogenic diet modified by replacing 6 per cent of the wheat gluten by wheat germ and giving 1 cc. of tomato juice per rat per day.

(c) (a) plus .00002 grams of potassium iodide.

(d) (a) plus .00128 grams of potassium iodide.

(e) (b) plus .00002 grams of potassium iodide.

(f) (b) plus .00128 grams of potassium iodide.

(g) The Steenbock rachitogenic diet with yellow corn in part replaced by from 12½ to 37½ per cent white bread, or 33 per cent rolled oats, or 33 per cent whole wheat, or 33 per cent rolled oats plus the ash from 33 per cent whole wheat, or 30 per cent non-irradiated whole wheat biscuits, or the ash from 10 to 20 per cent egg yolk, or 2.2 per cent chocolate, or the ash from 2.2 per cent chocolate.

(h) The Steenbock rachitogenic diet plus 1 to 2 per cent shortening.

TABLE II
EXPERIMENTAL ANIMALS ON ANTIRACHITOGENIC DIETS FOR FOUR WEEKS

Antirochitogenic diets	No. of animals	X-Ray	Blood[34] phosphorus mgs/100 cc.	Ash content of bones %	Non-hyperplastic %	Hyperplastic %	Thyroid Changes				
							Slight hyperplasia		Moderate hyperplasia	Marked hyperplasia	Degenerative
							Small acini	Large acini	Small acini Large acini	Small acini Large acini	
(a)	35	normal	3.8	44.2	0	51	17%		2%	32%	49%
							17%		2%	4% 28%	
(b)	8	normal	3.4	43.3	0	100	74%			26%	
							62%	12%		26%	
(c), (d)	4	normal	4.3	46.6	100	0					
(e), (f)	4	normal	4.5	46.2	100	0					
(g)	31	normal	3.1	44.9	32	68	56%		6%	6%	
							44%	12%	3% 3%	3% 3%	
(h)	12	normal	3.5	43.5	25	75	41%			34%	
							41%			17% 17%	

(i), (j)	28	normal	3.3	42.9	21	79	50%	3%	19%	7%
							50%	3%	19%	
(k), (l)	4	normal	4.7	41.9	100	0				
(m), (n)	4	normal	3.1	45.5	100	0				

The following antirachitogenic diets were used:

(a) The Steenbock rachitogenic diet plus from ½ to 1 per cent 1D Viosterol (irradiated ergosterol).*

(b) The Steenbock rachitogenic diet with yellow corn in part substituted by 30 per cent irradiated whole wheat biscuits.

(c) The Steenbock rachitogenic diet plus ½ per cent 1D Visoterol and .00002 grams of potassium iodide.

(d) The Steenbock rachitogenic diet modified by replacing 6 per cent of the wheat gluten by wheat germ and giving 1 cc. of tomato juice per rat per day, plus ½ per cent 1D Viosterol and .00002 grams of potassium iodide.

(e) The Steenbock rachitogenic diet plus ½ per cent 1D Viosterol and .00128 grams of potassium iodide.

(f) The Steenbock rachitogenic diet modified as in (d) plus ½ per cent 1D Viosterol and .00128 grams of potassium iodide.

(g) The Steenbock rachitogenic diet with yellow corn in part substituted by from 2 to 20 per cent egg yolk.

(h) The Steenbock rachitogenic diet with yellow corn in part substituted by the ash from 10 to 20 per cent egg yolk plus ½ per cent cod liver oil.

(i) The Steenbock rachitogenic diet modified as in (d) plus from ½ to 1 per cent cod liver oil.

(j) The Steenbock rachitogenic diet plus from ½ to 1 per cent cod liver oil.

(k) The Steenbock rachitogenic diet modified as in (d) plus ½ per cent cod liver oil and .00002 grams of potassium iodide.

(l) The Steenbock rachitogenic diet plus ½ per cent cod liver oil and .00002 grams of potassium iodide.

(m) The Steenbock rachitogenic diet modified as in (d) plus ½ per cent cod liver oil and .00128 grams of potassium iodide.

(n) The Steenbock rachitogenic diet plus ½ per cent cod liver oil and .00128 grams of potassium iodide.

* 1D Viosterol was obtained by diluting 250D Viosterol with the requisite amount of corn oil.

The markedly hyperplastic group was composed of glands in which were the most striking changes. Again, a decided variability in the size and shape of the acini was noted. Infoldings as well as cellular buds were numerous. To observe any colloid content was unusual. The vesicular epithelium tended to be columnar. The cytoplasm of the cells varied in the staining qualities from section to section. The enlarged, round or oval nuclei usually occupied a central position within the cell. Many mitoses were seen. The vascular spaces were engorged.

As well as the two divisions above described, reference must be made to a third condition, which, for purposes of classification, has been termed the degenerative group. The glands placed in this category presented alveoli which were irregular in contour and from which the colloid had almost entirely disappeared. The epithelial elements showed profound changes. The cells were enlarged often to a marked degree, having acidophilic cytoplasm and small round or irregular or fragmented, pyknotic, centrally placed nuclei. They appeared either discretely, compactly or in heaped up masses. Desquamation into the lumen was commonly encountered. Engorgement of the vascular bed was usually observed.

Results of Study

From the tables it will be seen that eighty-six experimental animals on the Steenbock rachitogenic diet, either unmodified or modified by the addition of vitamins other than vitamin D or with yellow corn in part substituted by a variety of substances, in 80 to 100 per cent, or, on the average, in 90 per cent of instances, showed a hyperplasia of the thyroid gland. This was slight in degree in 45 per cent, moderate in 36 per cent and marked in 9 per cent. Three per cent non-hyperplastic glands and 17 per cent showing degenerative changes were found in thirty-four animals on the unmodified Steenbock rachitogenic diet. In ten animals on the modified or unmodified Steenbock rachitogenic diet with added potassium iodide, the thyroid glands were non-hyperplastic.

Hyperplastic thyroid glands were found in an average of 75 per cent of forty-three animals on the Steenbock rachitogenic diet, unmodified or modified, with vitamin D added in the form of from 1/4 to 1 per cent 1D Viosterol, or with yellow corn in part substituted by irradiated whole wheat cereal biscuit. In 51 per cent of thirty-five animals on the Steenbock diet, with from 1/4 to 1 per cent 1D Viosterol added, thyroid hyperplasia was present. In 17 per cent of these it was slight, in 2 per cent moderate, and in 32 per cent marked. Forty-nine per cent of the thyroids of this group were placed in the degenerative class. In eight rats on either the modified Steenbock diet with 1/2 per cent 1D Viosterol added or with yellow corn replaced in part by irradiated whole wheat biscuits, 100 per cent had hyperplastic glands, the degree of which was slight in 74 per cent and marked in 26 per cent. On the Steenbock rachitogenic diet, modified or unmodified with 1/2 per cent 1D Viosterol to supply vitamin D, and potassium iodide added, eight animals had non-hyperplastic thyroid glands.

TABLE III

EXPERIMENTAL ANIMALS ON THE STEENBOCK RACHITOGENIC DIET FOR THREE WEEKS, FOLLOWED BY TEN DAYS ON VARIOUS TEST DIETS FOR THE PREVENTION OF RICKETS

Results of line test [17]	Number of animals	Blood[24] phosphorus mgs/100 cc.	Thyroid Changes						
			Non-hyperplastic %	Hyperplastic %	Slight hyperplasia	Moderate hyperplasia	Marked hyperplasia		Degenerative
					Small acini / Large acini	Small acini / Large acini	Small acini / Large acini		
1 plus healing	15	2.1	0	100	52%	40%	8%		
					26% 26%	20% 20%	8%		
2 plus healing	33	2.4	9	91	48%	34%	9%		
					30% 18%	18% 16%	9%		
3 plus healing	41	2.4	0	100	68%	26%	6%		
					49% 19%	12% 14%	6%		
4 plus healing	31	3.4	0	100	45%	42%	13%		
					32% 13%	13% 29%	13%		

TABLE IV

EXPERIMENTAL ANIMALS ON THE STEENBOCK RACHITOGENIC DIET CONTAINING VARYING AMOUNTS OF CALCIUM CARBONATE

Steenbock rachitogenic Diet cont. $CaCO_3$	No. of animals	Blood[24] phosphorus mgs/100 cc.	Ash content of bones %	X-Ray	Non-hyperplastic %	Hyperplastic %	Thyroid Changes				
							Slight hyperplasia	Moderate hyperplasia	Marked hyperplasia		Degenerative
							Small acini / Large acini	Small acini / Large acini	Small acini / Large acini		
3%	34	1.6	29.0	marked rickets	3	80	41%	30%	9%		17%
							29% 12%	9% 21%	9%		
½%	10	4.0	40.7	normal	0	100	20%	80%			
							10% 10%	60% 20%			
none	10	4.0	42.0	normal	0	100	56%	44%			
							45% 11%	44%			

When from 1/4 to 1 per cent cod liver oil, containing not more than .00125 to .005 mg. of iodine, was added per 100 grams to either the modified or the ummodified Steenbock diet, in twenty-eight animals non-hyperplastic glands were found in 21 per cent, and in 79 per cent hyperplastic glands. Of the latter 50 per cent were classified as having slight, 3 per cent moderate and 19 per cent marked changes. Seven per cent were of the degenerative type. Eight animals on similar rations with added potassium iodide had non-hyperplastic thyroid glands.

In thirty-one animals given the Steenbock diet, with from 10 to 20 per cent egg yolk replacing in part yellow corn, non-hyperplastic glands were obtained in 32 per cent, and 68 per cent were hyperplastic. Of these 56 per cent showed a slight grade of hyperplasia, and 6 per cent moderate, and 6 per cent marked degrees. When the ash from 10 to 20 per cent egg yolk was substituted and 1/2 per cent cod liver oil, containing approximately .0025 mg. of iodine, added to the Steenbock diet, in a group of twelve animals, non-hyperplastic glands were found in 25 per cent and hyperplastic glands in 75 per cent. Forty-one per cent of the latter organs presented slight changes and 34 per cent a marked degree of hyperplasia. These results indicated that a higher percentage of non-hyperplastic thyroid glands were obtained from animals whose diets contained egg yolk or the ash from egg yolk with added cod liver oil, than in other diets, except those to which potassium iodide had been added.

By combining the findings obtained at the end of the fourth experimental week on the Steenbock rachitogenic diet modified, unmodified, or with yellow corn substituted in part by a variety of substances, with or without added Viosterol, in 160 experimental animals, non-hyperplastic glands were present in 7 per cent, hyperplastic glands in 79.8 per cent and degenerative changes in 13.2 per cent. The degree of hyperplasia was slight in 47.4 per cent, moderate in 16 per cent and marked in 16.4 per cent. In 40 animals where cod liver oil replaced Viosterol in the diets, 23 per cent of the thyroid glands were non-hyperplastic and 73.5 per cent were hyperplastic. Hyperplasia was slight in 45.5 per cent, moderate in 1.5 per cent and marked in 26.5 per cent of these. Three and five-tenths per cent were of the degenerative type. With the addition of .00002 grams or .00128 grams of potassium iodide to the above diets non-hyperplastic thyroid glands were present in 100 per cent of twenty-six experimental animals.

One hundred and twenty experimental animals were placed for three weeks on the Steenbock rachitogenic diet, followed by ten days on various test diets for the prevention of rickets. These diets consisted of the Steenbock diet with 1/2 to 3 per cent 1D Viosterol, or 1/2 per cent shortening,

together with 1.5 per cent 1D Viosterol added, or with yellow corn in part substituted by 3.75 to 37.5 per cent white bread containing vitamin D.

Approximately 97 per cent of these animals had hyperplastic glands. The degree of hyperplasia was slight in 53 per cent, moderate in 35 per cent, and marked in 9 per cent. No regression in thyroid activity was demonstrable even with the greatest amount of bone healing.

When the above figures were combined with those previously indicated in the joint results obtained from the rachitic and antirachitic groups, exclusive of animals to whose diets cod liver oil or potassium iodide had been added, it was found that in two hundred and eighty experimental animals 4.6 per cent had non-hyperplastic thyroid glands, 88.4 per cent had hyperplastic organs, and 6.6 per cent of the glands were of the degenerative type. The degree of hyperplasia was slight in 50.2 per cent, moderate in 25.5 per cent and marked in 12.7 per cent.

The general nutrition of the animals was quite good. The average gain in weight of the animals on rachitogenic diets without additional iodine was 16 grams. When iodine was given the gain on the average was 20 grams. In animals on antirachitogenic diets, with no iodine supplemented, the gain was 24 grams. With additional iodine in antirachitic diets the gain was 31 grams. The greatest gain in weight, namely, 36 grams, was found in animals receiving egg yolk in their diet. Animals on the "line test" experiments gained least in weight. During the first three weeks very few showed any appreciable change. In the following ten-day period an average of 9 grams was gained.

The following observations were based on the study of one hundred and forty-six experimental animals, two from each group examined at weekly intervals. Hyperplastic changes in the thyroid glands began as early as the second experimental week and were consistently found to the end of the sixth week in animals placed at one month of age on the modified or the unmodified Steenbock rachitogenic diet, with or without added vitamin D, as 1/2 per cent 1D Viosterol. When .00002 gram of potassium iodide was added to the above diets the animals frequently showed slightly hyperplastic thyroid glands at the end of the third week, but from the fourth to the sixth week their glands were found to be non-hyperplastic. Non-hyperplastic thyroid glands were a constant finding in animals over a similar period to whose diets .00128 gram of potassium iodide had been added, instead of the smaller amount. Slightly hyperplastic glands were present in animals examined from the third to the seventh week when 1/2 per cent cod liver oil replaced 1/2 per cent 1D Viosterol in the diets. The thyroid glands from animals receiving .00002 or .00128

gram of potassium iodide in the cod liver oil containing diets were non-hyperplastic throughout a similar period.

Ten animals comprised a group on the Steenbock diet with 1/2 per cent calcium carbonate replacing the usual 3 per cent. At the end of four weeks all showed thyroid hyperplasia, the degree of which was slight in 20 per cent and moderate in 80 per cent. In smaller experimental groups receiving a diet in which from 1 to 2 per cent calcium carbonate was used, there was a gradual increase in the glands showing moderate hyperplasia. None of the animals receiving diets in which the calcium carbonate content was less than 3 per cent presented markedly hyperplastic glands.

In order to ascertain if the basal ration were capable of inducing thyroid hyperplasia merely due to its low iodine content, ten animals were placed on the Steenbock diet containing no calcium carbonate. After a month had elapsed all showed hyperplastic changes, the degree of which was slight in 56 per cent and moderate in 46 per cent.

The facts derived from the experimental results were that hyperplasia of the thyroid gland occurred in a high percentage of animals on the Steenbock rachitogenic diet with or without the addition of vitamin D in the form of Viosterol; that neither the supplying of an abundance of vitamins in the diets nor the replacement of a portion of the yellow corn by white bread, irradiated whole wheat cereal, rolled oats, whole wheat, rolled oats together with the ash from whole wheat, non-irradiated whole wheat cereal, egg yolk, the ash from egg yolk, chocolate, ashed chocolate, nor the addition of shortening to the diets, prevents its development; that approximately one-quarter of the experimental animals had non-hyperplastic glands when cod liver oil or an amount of egg yolk sufficient to prevent rickets, or the ash from egg yolk together with cod liver oil was added or substituted in the Steenbock rachitogenic diet; that no regression in hyperplasia was noted in animals despite a considerable amount of healing when test diets for the prevention of rickets were given for a ten-day period following the development of rickets; that the addition of .00002 gram or .00128 gram of potassium iodide to the Steenbock rachitogenic diet, either unmodified or modified, prevented thyroid hyperplasia at the end of four weeks; that .00002 gram of potassium iodide added to the modified or unmodified Steenbock diet was insufficient to prevent its occurrence entirely; that hyperplastic changes were frequently found in experimental animals having 1/2 per cent cod liver oil added to the Steenbock rachitogenic diet; that hyperplasia became manifest as early as the second week in animals receiving no additional iodine in their diets; that when the calcium carbonate content in the diet was reduced, a regression in the degree of

hyperplasia occurred; that hyperplasia occurred when no calcium carbonate was added to the Steenbock diet.

Discussion

Infections, intoxications, confinement, deficiencies, and excesses in food intake have been considered to influence the thyroid gland. Unrecognized agents, which in animals living in their natural environment have little effect, might exert powerful stimuli under experimental conditions. Although experimentally more than a single factor may be involved, only the most outstanding have been considered to be responsible for the manifestations. In view of our limited knowledge it would be futile to attempt to make finer distinctions at the present time.

Of the deficiencies affecting the thyroid gland the lack of iodine is well known. Most authors are agreed that in its absence pathological changes are evidenced in enlargement with hyperplasia. The results of partial deficiencies are less clearly defined. In their presence the effect of further stimulation from some other source would probably give rise to a greater response than when an adequate amount of iodine is available.

Hayden, Wenner and Rucker (4), and McClendon and Williams (16), produced thyroid enlargement in rats on diets having an iodine content of only 9 to 10 parts per billion. Tanabe (23) found thyroid hyperplasia in rats receiving a diet with low iodine content. In view of these observations, the finding of enlargement with hyperplasia in rats on the Steenbock rachitogenic diet was to be expected, since this diet obviously must have a low and variable iodine content, depending on the amount of the element in the soil where the grain has been grown (15). The giving of City of Toronto water, having an iodine content of 1.45 parts per billion (15), was insufficient to overcome this deficiency.

McCarrison (10) has stated that goitre in wild rats from different regions of India is excessively rare. For this reason, the finding of a high incidence of enlarged hyperplastic glands in the series of wild rats from this city was considered of interest. Toronto, Ontario, is situated in the endemic zone of the basin of the Great Lakes, where the existence of an iodine deficiency is well known (17).

Marine and Kimball (9) have shown that giving a small amount of any salt of iodine protects against simple goitre in lower animals and in man. McClendon and Williams (16), and Krause and Monroe (7) have found that the addition of iodine to diets low in this constitutent produced smaller thyroid glands in rats. McCarrison (11) noted in rats and pigeons an increase in the colloid content of the thyroid when iodine was given with

a mixed diet. These observations receive further substantiation by the finding of non-hyperplastic thyroid glands in animals on diets capable of producing thyroid hyperplasia when supplemented by small amounts of potassium iodide.

According to the observations of E. and M. Mellanby (18), normal glands were found when cod liver oil was added to diets producing thyroid changes in dogs. Luce (8) and McCarrison (12–14) have reported the presence of normal glands in rats receiving different diets containing not more than 2.5 per cent cod liver oil. In the present study the amount of cod liver oil added was from one-quarter to one per cent. In animals receiving 1 per cent cod liver oil, non-hyperplastic and slightly hyperplastic glands were found, whereas those obtaining lesser amounts presented hyperplastic changes frequently to a marked degree. It would seem necessary, therefore, to add more than 1 per cent cod liver oil, containing approximately .000005 gram of iodine, to the Steenbock diet, if the production of the non-hyperplastic state is to be assured.

The occurrence of thyroid hyperplasia in dogs and rats on rachitic diets has been noted by a number of investigators, but has not been considered as the direct result of a deficiency of the antirachitic vitamin. However, that vitamins may play a part in this phenomenon has received consideration by McCarrison (12). He has been able to exclude vitamin D as a factor by the finding of goitre in rats receiving a sufficiency through exposure to direct sunlight. Krause and Monroe (7) obtained enlarged thyroid glands in rats after four weeks on the Steenbock rachitogenic diet. When ultraviolet radiation for the following four weeks was given, no decrease in size of the thyroid occurred unless potassium iodide was added to the diet. Their observations are in accord with the results of the present study, since enlargement with hyperplasia was found in experimental animals on the Steenbock rachitogenic diet or when vitamin D, in the form of Viosterol, was added to it. Hyperplastic changes were not shown by animals receiving sufficient iodine for their needs in the absence of the antirachitic vitamin.

Clinically, rickets, osteomalacia, and osteoporosis associated with goitre have been frequently reported in man and animals (6–9). The investigations of Parhon (20) and of Aub, Bauer, Heath and Ropes (1) indicated that the thyroid exerts an influence on the calcium metabolism, since they found that the calcium excretion could be greatly increased by means of the administration of thyroid extract. This observation received further substantiation by the finding of an excessive excretion of calcium in cases of hyperthyroidism and decalcification of bones in cases of long duration.

It has, furthermore, been shown that the laying down of calcium was decreased when the thyroid was removed and increased when thyroid was given. Although these studies suggest that there is a relationship, the manner in which this is effected is purely speculative.

Tanabe (23) found that the addition of 2 per cent calcium chloride enhanced the degree of hyperplasia occurring in rats on a low iodine-containing diet. While Hellwig (5) was unable to reproduce the slight hyperplastic changes on a similar basic ration, he found that hyperplasia was present when 2 per cent calcium chloride was added to it. The addition of 0.41 to 0.59 per cent calcium carbonate to a mixed diet increased the colloid storage in both rats and pigeons according to McCarrison (11). Supplementing iodine in high calcium diets, according to these investigators, produced relatively normal glands. Luce (8) noted the occurrence of normal thyroid glands in rats on diets containing 0.25 or 0.04 per cent calcium. Murray observed acute hyperplasia in the thyroid glands of dogs on rachitic diets, either calcium-free or with added calcium. Sorour (22), using diets containing 3 per cent calcium and .01 per cent iron, found hyperplasia of the thyroid glands of rats kept in darkness. The results suggest that hyperplasia will likely be produced when a diet of low iodine content contains as high an amount of calcium as 2 per cent. This deduction finds support in the initiation of thyroid hyperplasia in animals receiving the various modifications of the Steenbock rachitogenic diet without supplemental iodine all of which contained 3 per cent calcium carbonate.

The question of the relation between the amount of calcium in the diet and the degree of hyperplasia has to be considered. The results of study indicated that as the calcium carbonate content of the diet was decreased, a gradual dimunition was apparent in the hyperplastic process. This transition may be further exemplified by comparing the degree of hyperplasia found when the Steenbock diet was given and when 1/2 per cent calcium carbonate replaced the customary 3 per cent. In the former instance 41 per cent were classified as slight, 30 per cent as moderate and 9 per cent marked hyperplasia, while in the latter 20 per cent showed slight changes and 80 per cent a moderate degree of hyperplasia.

In the present study thyroid hyperplasia in rachitic and non-rachitic rats was the result of an iodine deficiency, together with an excess of calcium in their diets. To secure non-hyperplastic glands there needs must be a favorable ratio between the iodine and the calcium in the intake. The presence of thyroid hyperplasia in rats on the Steenbock diet adds an avoidable complication which should be considered. In view of the fact that calcium is withdrawn from the bones in hyperthyroidism (1), and

although such a condition might actually favor the development of rickets, if one were testing various curative diets, a hyperfunctioning gland might actually deter the healing process.

The presence of degenerative changes in some of the glands adds a further complication. Although the highest incidence appeared in the only group which was not obtained immediately following exsanguination, to relegate these entirely to post mortem effects seems unjustifiable, since similar observations were also made in other groups. The explanation may be in part a greater tendency for the enlarging epithelial cells to shrink in fixative at a particular developmental period, as well as to become detached more easily when the vesicular content is less dense.

Variations in the degree of thyroid hyperplasia were always found within an experimental group. Why this should have occurred is difficult to explain. Such factors as animal susceptibility, season of the year, minor differences in the food from time to time (e.g., new grains, etc.), unrecognized intercurrent infections, the intake, the size of the animal, all exert an influence in any biological test. When one is using such a small experimental animal as the rat, very minute changes needs must be considered. It is only when a particular observation has been made sufficiently often that a justifiable conclusion may be drawn from animal experimentation. For this reason, the present report, which is based on the study of a large number of animals, represents significant results.

In conclusion, I wish to thank Professor Alan Brown, Doctor F. F. Tisdall, and Doctor T. G. H. Drake for making available their experimental material for pathological study, and to express my gratitude to Miss Ruth Herbert for the excellent care taken of the animals as well as for the chemical determinations.

Summary

1. The thyroid glands from 385 rats have been studied.

2. Diffuse hyperplasia was produced in rats through the feeding of the Steenbock rachitogenic diet or its various modifications.

3. Hyperplasia developed in the presence or absence of the antirachitic vitamin.

4. The factors underlying the development of hyperplasia in experimental animals on these diets were a deficiency of iodine, associated with an excessive amount of calcium carbonate.

5. Increasing the amount of calcium carbonate in diets deficient in iodine resulted in enhancement of the degree of hyperplasia.

6. Variations in the degree of hyperplasia were found within similar experimental groups.

7. The addition of a small amount of potassium iodide to the diets prevented the development of hyperplasia.

8. A high incidence of goitre was found in a series of wild rats from this city.

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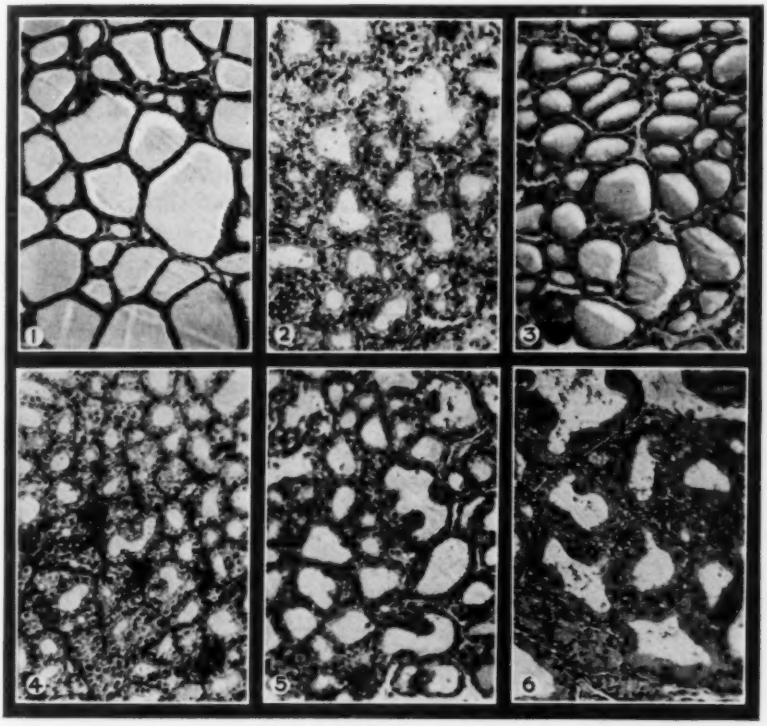

DESCRIPTION OF PLATE

FIG. 1. Normal thyroid of untreated wild rat.
FIG. 2. Idiopathic hyperplasia of thyroid in untreated wild rat.
FIG. 3. Non-hyperplastic thyroid of rats receiving iodine.
FIG. 4. Thyroid showing slight hyperplasia; low iodine, high calcium diet.
FIG. 5. Thyroid showing moderate hyperplasia; low iodine, high calcium diet.
FIG. 6. Thyroid showing marked hyperplasia; low iodine, high calcium diet.

THE UTILIZATION OF CALCIUM IN SOY BEAN DIETS

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IN THE Occident a calcium deficiency is frequently found in diets regarded as adequate in other respects. In China the problem of an adequate supply of calcium is even more urgent, although a lack in calcium may also be accompanied by deficiencies in both protein and calorific intake. Cows' milk, upon which the people of western countries depend in large part for their calcium intake, does not figure seriously in the dietary of China. A dairy industry in China is essentially non-existent and at present is economically impossible. Moreover, the cost of raw milk, or of imported milk, either in tins or in the form of dried powder, is far beyond the reach of the average food purse. The dangers of calcium deficiency are somewhat mitigated by the fact that breast feeding is continued till the infant is three or four years old, but for the remainder of the growth period and for the entire period of adult maintenance the individual is dependent upon calcium derived from vegetable sources. A very considerable amount of this calcium is supplied by soy bean products. Soy bean curd (soy bean cheese) would seem to have filled, in part, the place which milk has occupied in the Occident. Recent years have seen an extended use of the closely related food product, soy bean milk, which has been successfully applied to infant feeding.

Unpublished data gathered in this laboratory indicate that the daily average calcium intake for the adult of 55 to 60 kilograms body weight in north China is 0.39 gram with a protein intake of 70 to 80 grams. The accepted figure for the minimum calcium requirement in the Occident is that of Sherman (1), 0.45 gram per day for an adult of 70 kilogram body weight, with a minimum protein requirement of 44 grams. To provide a margin of safety it has been suggested that the actual calcium intake should be between 0.7 and 1.0 gram per day. The average calcium intake of the adult in America is approximately 0.75 gram per day; the 0.39 gram calcium per day recorded for north China provides no such margin of safety.

Little is known regarding the degree to which the calcium of soy bean curd is utilized by the animal organism. Tso (2) has shown that calcium storage can be secured with infants fed soy bean milk. There is a certain

amount of evidence supporting the view that calcium proteinate is more easily absorbed than simple salts. But there is no general agreement on the extent to which vegetable calcium is assimilated by the body when compared, for example, with the calcium from milk. McClugage and Mendel (3) report that the calcium of spinach and carrots is poorly assimilated by dogs, and Boas (4) reported that spinach fed to rats was not favorable to calcium retention. Sherman and Hadley (5) found that in children the calcium of milk is more efficiently utilized than vegetable calcium. Blatherwick and Long (6) on the other hand, feeding cabbage, celery, and such vegetables, and Rose (7), experimenting with carrots, assert that calcium from vegetable sources is quite equal to the calcium of milk in the degree to which it is utilized in human adult maintenance.

The experiments here reported were planned for the purpose of determining the extent to which the adult can utilize the calcium of soy bean curd. Moreover, for the purpose of making a comparison between the effect of high and low protein intake on calcium utilization, two experiments were carried out. In one of these experiments, the protein intake was fixed at the more luxurious level of 65 to 90 grams per day. In the other experiment, the daily intake was 35 to 45 grams of protein, which, while admittedly low, provides a typical example of restricted protein intake, such as is not infrequently met with in the Orient.

Experimental

Three normal adults, Chinese, ages 18 to 27, ingested a typical north China diet so arranged that about 80 per cent of the calcium was furnished by soy bean curd. In a corresponding experimental period using the same diet, soy bean curd was replaced by milk as the source of calcium. Two of the subjects W.B.C. and T.L.W., both men, were laboratory technicians; the third S.C.C., a woman, was a member of the laboratory instructional staff. Two experiments were conducted at an interval of three months. Experiment 1 provided for a low protein intake; Experiment 2 provided a more liberal protein intake. The calcium intake in both experiments was set at 0.45 gram per day, this figure approximating the minimum calcium requirement, for it was presumed that differences in calcium availability would be more evident at a level approaching this value. Each experiment consisted of two experimental periods of seven days each, one following immediately upon the other; one period involved the ingestion of soy bean curd and the other the ingestion of milk as the principle source of calcium. The order of these two periods was reversed in the two experiments.

The foods selected for consumption were in general characteristic of the

Chinese diet, except that with all three subjects the consumption of milk was a distinct innovation. The milk was brought to the boiling temperature and ingested warm. The soy bean curd was sometimes cooked with cabbage and sometimes fried lightly in oil. Cornstarch and sugar are not common constituents of the diet, but small amounts were added in these experiments to increase the fuel value of the daily ration.

The food was prepared and eaten in the laboratory. The soy bean curd used was the variety manufactured by coagulation with salt bittern, the so-called northern bean curd, and was purchased from a local soy bean shop. The cows' milk was supplied by the local dairy. The soy bean curd and cows' milk were delivered daily. The bread was the typical steamed bread of north China made from white flour. The other foods were such as could be purchased in quantity and kept for the length of the experiment. The amounts of food consumed daily throughout the seven days of each period are recorded in Table I. The total calorific value of the diet is

TABLE I

DAILY FOOD INTAKE—WEIGHTS IN GRAMS

Subject	Low Protein Diet				High Protein Diet			
	Milk		Soybean Curd		Milk		Soybean Curd	
	S.C.C.	W.B.C. T.L.W.	S.C.C.	W.B.C. T.L.W.	S.C.C.	W.B.C. T.L.W.	S.C.C.	W.B.C. T.L.W.
Food materials								
Milk.....	300	300	—	—	290	290	—	—
Soy bean curd	—	—	230	230	—	—	210	210
Rice.....	140	140	140	140	280	110	320	110
Bread.....	80	80	80	80	70	600	20	600
Meat.....	50	50	50	50	150	100	100	100
Fat.....	50	50	50	50	20	20	20	20
Cabbage....	100	100	100	100	50	—	50	—
Cornstarch...	—	55	—	55	—	36	—	31
Sugar.....	50	95	50	95	20	35	10	30
Total calories...	1833	2233	1778	2178	2142	2845	1897	2744
Total protein....	36.76	36.76	44.00	44.00	62.24	83.83	61.63	92.15
Total phosphorus	.665	.665	.616	.616	1.040	.822	.888	.793
Total calcium...	.448	.448	.449	.449	.443	.456	.442	.456
Per cent calcium furnished by:								
Milk.....	80.3	80.3			78.5	76.3		
Soy bean curd			80.9	80.9			78.5	76.3

calculated in the usual way. As a matter of interest, the total phosphorus content calculated from standard analyses is included also, although the experiment was directed solely to the question of calcium metabolism. The subjects ate three meals a day; the milk and soy bean curd in their respective periods were consumed, the milk at breakfast, the bean curd at the noon and evening meals. Distilled water only was used for drinking and in preparation of the food. No tea was drunk during the period of the experiment.

Calcium and nitrogen analyses were carried out on the daily samples of milk and bean curd and on each new lot of the other food materials as purchased; the usual care was taken in securing uniform samples. The averages of these analyses are indicated in Table II.

TABLE II

AVERAGE NITROGEN AND CALCIUM CONTENT OF FOOD MATERIALS CONSUMED
(in percentage of edible portion)

	Nitrogen	Calcium
Milk	0.528	0.120
Soy bean curd	1.28	0.162
Rice	1.28	0.018
Bread	1.32	0.013
Meat	2.56	0.007
Cabbage	0.17	0.049

Urine and feces were collected for the last four days of each 7-day metabolism period. The urine was collected and analyzed in 24-hour samples. The feces were collected as a composite for the four days, thoroughly mixed, and aliquots taken for analysis. Charcoal was used in marking off the feces for the different periods. The calcium in food, feces, and urine was determined by McCrudden's method (8). Nitrogen in the food and feces was determined by the Kjeldahl method; for nitrogen in urine, the micromethod of Folin and Denis (9) was employed.

RESULTS

Tables III and IV give the calcium and nitrogen balances for the two experiments. In Table V, the balances are summarized in milligrams per day per kilogram of body weight. The low protein diet furnished an amount of protein which was quite insufficient for these subjects and negative nitrogen balances resulted.

TABLE III
NITROGEN AND CALCIUM BALANCES
First Experiment—Low Protein Diet
(in grams per four-day period.)

Subject weight	Diet	Nitrogen				Calcium			
		Intake	Urine	Feces	Balance	Intake	Urine	Feces	Balance
S.C.C.	Milk	23.52	31.28	2.91	−10.67	1.792	0.395	2.120	−0.723
55.2 kilos	Soy bean curd	28.16	28.48	3.42	−3.74	1.796	0.348	1.986	−0.538
W.B.C.	Milk	23.52	33.30	3.46	−13.24	1.792	0.419	2.380	−1.007
54.0 kilos	Soy bean curd	28.16	32.32	7.46	−11.62	1.796	0.516	1.838	−0.558
T.L.W.	Milk	23.52	31.30	4.23	−12.01	1.792	0.373	1.658	−0.239
50.0 kilos	Soy bean curd	28.16	27.84	4.85	−4.53	1.796	0.469	1.496	−0.169

TABLE IV
NITROGEN AND CALCIUM BALANCES
Second Experiment—High Protein Diet.
(in grams per four-day period.)

Subject weight	Diet	pH of Urine	Nitrogen				Calcium			
			Intake	Urine	Feces	Balance	Intake	Urine	Feces	Balance
S.C.C.	Soy bean curd	6.0	40.44	40.24	5.00	−4.80	1.768	0.548	1.288	−0.068
55.0 kilos	Milk	6.1	39.83	41.65	3.87	−5.69	1.772	0.508	1.211	+0.053
W.B.C.	Soy bean curd	6.0	58.96	42.40	6.84	+9.72	1.824	0.368	1.206	+0.250
55.7 kilos	Milk	6.0	53.64	45.40	5.62	+2.62	1.824	0.381	1.104	+0.339
T.L.W.	Soy bean curd	6.3	58.96	45.62	6.41	+6.93	1.824	0.463	1.389	−0.028
55.0 kilos	Milk	6.4	53.64	44.17	6.29	+3.18	1.824	0.382	1.597	−0.155

The subjects in the experiments were not accustomed to consuming such a large quantity of milk, somewhat over half a pint per day! They found the milk diet somewhat constipating, while the soy bean diet just as distinctively possessed the laxative properties of the usual Chinese diet.

TABLE V

NITROGEN AND CALCIUM BALANCES

(in milligrams per day per kilogram of body weight)

Diet	Nitrogen balance		Calcium balance	
	Milk	Soy bean	Milk	Soy bean
Exp. 1: *Low protein intake*				
Subject:				
S.C.C.	−48.0	−17.0	−3.3	−2.5
W.B.C.	−61.0	−54.0	−4.7	−2.6
T.L.W.	−60.0	−23.0	−1.2	−0.8
Average:			−3.1	−2.0
Exp. 2: *High protein intake*				
Subject:				
S.C.C.	−26.0	−22.0	+0.2	−0.3
W.B.C.	+12.0	+44.0	+1.5	+1.1
T.L.W.	+14.0	+31.0	−0.7	−0.1
Average:			+0.3	+0.2

Examination of the calcium balance figures indicates that there was no significant difference in the extent to which the calcium furnished by cows' milk and soy bean curd was assimilated. As to the amount of calcium ingested, it would seem that 0.45 gram per day is the very minimum possible on the type of diets here employed. In the second experiment, a more favorable calcium balance accompanied a liberal protein intake.

The results in these experiments are not complicated by great differences in the acid-base balance of the milk and soy bean curd diets. Computing the acid-base residues from the figures of Sherman and Gettler (10) for milk, and from our own for soy bean curd, it was found that the soy bean curd was slightly more basic than milk, to an amount equivalent to 0.5 cc. of normal base per 100 grams of the food material. This difference is too slight to cause any material variation in the retention of calcium in the two periods. As a check upon the acid-base balance, the pH of the urine of all three subjects during the second experiment, was determined colorimetrically each day. The pH of each of the subjects remained practically constant for the four days; the average values are shown in Table IV.

In experimenting with Chinese diets, the factor of bulk is usually a pronounced one. The diets consumed in these experiments were not characterized by excessive bulk, since it was necessary to avoid the usual large amounts of calcium-rich vegetables, such as cabbage, turnips, etc. The effect of bulk in the Chinese dietary on the assimilation of calcium remains to be studied.

Summary

Metabolism experiments were conducted for the purpose of comparing the utilization by adult man of the calcium furnished by cows' milk and soy bean curd (soy bean cheese).

The calcium balances indicate that cows' milk and soy bean curd are equally effective as sources of calcium in the Chinese diet.

Increasing the protein intake facilitated the attainment of calcium equilibrium.

It is shown that a figure approaching 0.45 gram of calcium per day may be regarded as the minimum daily requirement for the Chinese adult of 55 kilograms body weight.

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THE HEAT PRODUCTION OF CATTLE IN A RESPIRATION CALORIMETER AS RELATED TO THE RATE OF VENTILATION AND TO THE MOISTURE CONTENT OF THE AIR

By

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THE subject of this discussion is significant in relation to studies of the heat production of cattle as the rate of ventilation and the moisture content of the ingoing air of the respiration chamber, or calorimeter, affect the moisture and carbon dioxide contents of the air within the apparatus, also the proportionate heat outgo by radiation and as latent heat of water vapor, and, finally, the total heat outgo from the animal body.

This is a matter of especial concern in indirect calorimetry, by the respiratory quotient procedure, since in the use of this method it is desirable so to restrict the rate of ventilation that the carbon dioxide and the oxygen contents of the outcoming air may differ as much as practicable from these measurements of the ingoing air—in order that the elements of error in analytical work may be acceptably small in comparison with the observations sought. The results of this study are also of interest because of their implications in relation to prevailing standards of barn ventilation.

The plan of this experiment, as outlined in Table I, provided for two series of four one-day heat measurements each, on a dry cow as the experimental subject. The ration was the same, throughout, and provided three-fourths of the maintenance requirement of energy. The rate of ventilation was alternately fast and slow on successive days in both series of observations; and the greater part of the moisture was frozen out of the incoming air during the second of the two series of heat measurements.

The slower rate of ventilation was approximately 15 per cent of the rapid rate, which latter was the rate ordinarily employed in direct calorimetric experimentation with cattle at this Institute.

In both series of observations intermediate periods of eight hours each, during which the rates of ventilation were maintained as in the observa-

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tion periods to follow, were interposed between intervals of heat measurement; hence the experimental days do not coincide with calendar days.

TABLE I
SCHEDULE OF EXPERIMENTATION

Period No.	Treatment	Intervals of Experimentation	Day of observation	Ventilation per hour
				Liters
1	Air contained natural moisture	12 hours prel.		
		24 hours heat meas.	1	35,120
		8 hours intermed.		
		24 hours heat meas.	2	5,454
		8 hours intermed.		
		24 hours heat meas.	3	35,656
		8 hours intermed.		
		24 hours heat meas.	4	5,449
2	Air freed from moisture by freezing	12 hours prel.		
		24 hours heat meas.	1	35,096
		8 hours intermed.		
		24 hours heat meas.	2	5,414
		8 hours intermed.		
		24 hours heat meas.	3	35,066
		8 hours intermed.		
		24 hours heat meas.	4	5,419

The purpose of these intermediate intervals was to establish constant conditions—of the animal, of the air in the chamber, and of the chamber itself—representative of the rate of ventilation to follow.

The animal was fed twice in each twenty-four hour measurement interval, the feedings being twelve hours apart; and the last feeding before the beginning of these twenty-four hour heat measurement periods took place four, eight, or twelve hours previously—on the different days. It seems unlikely, therefore, that the heat-stimulating effect of feeding, and of the early hours of digestion, could have contributed an uneven influence of important magnitude in the measurements of heat production.

The daily ration consisted—in terms of dry matter—of 1744 grams of alfalfa hay and 1821 grams of grain, the latter being a mixture devised for another purpose and containing 23.53 per cent wheat germ, 17.65 per cent of distiller's grains, 23.53 per cent corn meal, 17.65 per cent ground oats, 11.76 per cent ground barley, and 5.88 per cent linseed meal.

The water consumption of the cow during the first four-day period was 18.055, 15.055, 17.985, and 13.605 kgm. on successive days, and during the second four-day period was 12.920, 13.575, 15.730 and 9.495 kgm.,

likewise on successive days. The cow was watered twice daily, but inasmuch as the quantity consumed naturally varied much, from day to day, the foregoing weights of water drunk are not closely significant in relation to the differences in treatment.

The average weight of the cow in period 1 was 450.2 kgm. and in period 2 was 449.9 kgm.

The temperature of the air in the calorimeter was maintained constant within 0.2 degree Centigrade, the average being 21.8°.

In Table II are given the data for heat emission, at the different rates of ventilation, in relation to the moisture of the air and to the water vaporized,—the heat being measured by direct calorimetry.

In period 1 the relative humidity of the ingoing air naturally varied with the weather, and, on the average, 85 per cent of this moisture was frozen out during period 2.

The relative humidity of the air in the chamber varied, as would be expected, with the rate of ventilation, and the air which was dried by freezing did not carry out of the chamber quite as much moisture as did that which entered the calorimeter with its natural moisture content.

The data for latent heat of the water vapor measured as such (third column of figures) show that the dried air, and the rapidly circulating air, took up a greater quantity of water than did the humid and slow moving air.

In order to obtain measures of the total heat carried away from the animal as latent heat of water vapor, the latent heat of the water measured as vapor was added to the heat of condensation of the water which formed on the cooling system of the calorimeter.

These data for latent heat of total water (fifth column of figures) naturally varied from high to low values directly as the rate of ventilation.

The two columns of figures at the right of Table II give the percentages of the total heat emission eliminated as latent heat of water vapor, with and without corrections for the quantities of heat liberated on condensation of water on the cooling system of the calorimeter, the data including this correction being given in the right-hand column of the table.

These data show that approximately 40 per cent of the heat production left the animal as latent heat of water vapor, and, in general (one exception), that with decreased rate of ventilation there was decrease in the percentage of heat leaving the body as latent heat of water vapor.

In Table III are presented the data relating the rates of ventilation to the heat production, the rapid rate being such as to give, in the outcoming air, an average CO_2 content of 0.304 per cent, and the slow rate an average

TABLE II

RELATIONS OF THE HEAT PRODUCTION OF A COW TO THE MOISTURE OF THE AIR AND TO WATER VAPORIZED

Treatment	Day of observation	Rate of ventilation	Rel. humid. ingoing air	Rel. humid. air in chamber	Latent heat of water vapor	Heat of condensation of water	Latent heat of total water	Total heat emission	Latent heat of water vapor; per cent of total heat	
									Not corrected for cond. of water	Corrected for cond. of water
			Per cent	Per cent	Cals.	Cals.	Cals.	Cals.		
Period 1: Air contained natural moisture	1	Rapid	23.7	68.4	4180	772	4952	12,447	33.6	39.8
	2	Slow	18.4	87.3	1000	3571	4571	11,420	8.8	40.0
	3	Rapid	21.6	66.3	4254	694	4948	11,735	36.3	42.2
	4	Slow	42.2	90.0	693	3807	4500	11,495	6.0	39.1
Period 2: Air freed from moisture by freezing	1	Rapid	3.7	54.7	4723	0	4723	11,524	41.1	41.1
	2	Slow	4.7	84.2	1142	3380	4521	11,709	9.8	38.7
	3	Rapid	3.2	58.9	5204	287	5491	11,919	43.8	46.1
	4	Slow	4.7	86.8	1177	3665	4842	11,937	9.9	40.6

TABLE III

HEAT PRODUCTION OF A COW AS RELATED TO THE RATE OF VENTILATION IN A RESPIRATION CALORIMETER

Treatment	Day of observation	Rate of ventilation	CO_2 in outcoming air	Total heat production*	Time spent standing	Observed heat production corrected to standard day	Computed heat production corrected to standard day	Computed heat ÷ observed heat
			Per cent	Cals.	Hours	Cals.	Cals.	Per cent
Period 1: Air contained natural moisture	1	Rapid	0.312	12,523	15.9	12,242	12,329	100.7
	2	Slow	1.673	11,460	9.1	11,669	11,669	100.0
	3	Rapid	0.299	11,736	10.3	11,859	12,228	103.1
	4	Slow	1.711	11,435	8.9	11,657	11,799	101.2
Period 2: Air freed from moisture by freezing	1	Rapid	0.298	11,490	10.7	11,584	11,910	102.8
	2	Slow	1.763	11,643	14.2	11,485	11,695	101.8
	3	Rapid	0.307	11,905	13.6	11,790	12,113	102.7
	4	Slow	1.752	11,825	14.0	11,682	11,894	101.8

* Corrected for heat stored in animal body.

CO_2 content of 1.725 per cent—the higher concentration being 5.67 times the lower. A CO_2 content of 1.0 per cent, in the outcoming air, is satisfactorily high for purposes of respiratory quotient calorimetry.

The total heat production as given in this table is derived from the heat emission, as stated in Table II, by correction for heat stored in the animal body. For the purpose of this study, however, it is necessary to compute, or to correct, the heat production to represent a standard day as to standing and lying, thus establishing a much more satisfactory basis of comparison of the treatments to which the subject was subjected.

This correction was made by employing the factor 16.0 Calories to represent the greater energy expense, per 100 kgm. live weight, of standing as compared with lying. This factor is the average of five determinations (15.1, 15.1, 16.9, 14.0 and 18.9 Cals.) with steers Nos. 47, 36, 57, 17, and 85, respectively, in experiments Nos. 238, (1) 240, (2) and 241 (3).

The heat production thus corrected is given in the second and third columns of figures from the right of this table, based in one case on the direct heat measurement and in the other on the heat as computed by indirect calorimetry.

In computing the heat production for period 1, by the indirect procedure, it was necessary to assume that the methane production for each day was the average of the values for daily methane production as determined in period 2, since methane was not determined in the first period. The justification for this procedure is that the same experimental subject and the same quantity of the same ration were involved.

The column at the right of Table III shows that the heat production was satisfactorily measured by both of the procedures employed, the maximum difference between the data for any one day's measurement by the two methods being 3.1 per cent.

This series of measurements of the heat production of a cow during eight successive days, separated by eight-hour intervals, would be considered as fairly satisfactory repeats if all represented the same conditions.

That the heat production of the first day was definitely higher than that of the second day could be plausibly explained as resulting from the fact that this was the first day that the cow had spent in the calorimeter; and it is common under these conditions for the heat production to be somewhat elevated; also, the animal changed position—as to standing and lying—more frequently on the first day than on any subsequent day.

From these data it is clear that the great differences which prevailed in the rate of ventilation produced no marked differences in the total heat production.

It is true, however, that with each change in the rate of ventilation from rapid to slow there was a slight decrease in the heat production; and, conversely, that with each change in the rate of ventilation from slow to rapid there was a slight increase in the heat production.

In view of the fact that the foregoing observation holds true in all of the six changes in the rate of ventilation, as indicated by both the direct and the indirect methods of measurement of the heat production, it is clear that with the slow rate of ventilation there is a slight but relatively unimportant decrease in the heat production. The writers suggest that the most likely cause of this effect is a decrease in the voluntary activity of the animal.

Attention is called to the facts that in the present experiment the ration was sufficient to supply only three-fourths of the maintenance requirement of energy; that the calorimeter chamber was maintained at a temperature of 21.8°C; and that the chamber was efficiently cooled by water circulating inside it.

With full feeding of the experimental subject the heat production would have been much higher; and in the use of respiration chambers in warm weather the temperature of the air might be higher than it was during this experiment. Under such circumstances, therefore, an adequate cooling device would probably be required, in connection with the respiration chamber, in order to prevent discomfort of the animal and disturbance of its heat production.

Summary

The heat production of a dry cow, on a constant ration, was measured in a respiration calorimeter by direct and also by indirect calorimetry, in eight consecutive twenty-four hour intervals of observation, to determine the effects of the rate of ventilation of the calorimeter on the paths of outgo of heat from the animal and on the quantity of the total heat produced.

The rate of ventilation was rapid or slow in alternate twenty-four hour intervals, the rapid rate being about 35,000 liters per hour, and the slow rate from 5,414 to 5,454 liters per hour; and in the second four of the eight intervals of observation about 85 per cent of the moisture was removed from the ingoing air by freezing.

The carbon dioxide content of the outcoming air at the slow rate of ventilation was 10.3 times as great, and at the high rate of ventilation 1.8 times as great, as that permitted by King's standard (4) (.167 per cent) for barn ventilation.

The moisture content of the outcoming air at the slow rate of ventila-

tion was 1.4 times as great as at the rapid rate,—a large proportion of the moisture given off by the animal having been condensed on the cooling system of the calorimeter.

With both methods of determination of the heat production there was, with each change in the rate of ventilation from rapid to slow, a slight decrease in the heat production; and with each change from slow to rapid, a slight increase in the heat production.

Approximately 40 per cent of the heat produced was eliminated by the animal as latent heat of water vapor, the quantity of which generally decreased to a slight extent with decrease in the rate of ventilation.

Inasmuch as the carbon dioxide content of the outcoming air at the slow rate of ventilation averaged 1.725 per cent, whereas a carbon dioxide content of 1.0 per cent is ample for satisfactory respiratory quotient calorimetry, the authors conclude that it is unlikely that the rate of ventilation would affect the heat production in respiratory quotient calorimetry if the respiration chamber is equipped with an adequate cooling device.

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THE ECONOMY OF CONVERSION OF FOOD ENERGY INTO MILK ENERGY BY THE DAIRY COW

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A SIGNIFICANT consideration in relation to milk production as a factor in systems of agriculture is the fundamental economy with which the cow transforms her own feed into food available for human beings—as measured by the proportions of the food energy which the cow turns into milk.

An opportunity to make observations on this matter having arisen in connection with a series of year-long mineral balance studies, the results of this incidental study are here separately presented.

One point of view from which this matter has already been studied is that of the relative economy of utilization of metabolizable energy for the maintenance, body increase, and milk production of cattle.

Thus, Forbes, Fries, Braman and Kriss (1) found the average rates of utilization of metabolizable energy for maintenance, lactation and body increase to be as 1.0 for maintenance, 0.985 for lactation, and 0.761 for body increase.

In the present study, however, this point of view is precluded by the fact that the plan of experimentation provided no basis for determination of the maintenance requirement of energy.

At the same time a broadly significant and practical consideration in this relation is that of the gross return of milk energy from a year's investment of food energy, without separation of the quotas for maintenance and actual production; and this is the point of view of the present study.

The results of this study, therefore, apply with exactness only to cows of the particular size and productive capacity of the subjects of this investigation; but inasmuch as these cows represented moderately high normals—well above the commercial average—the conclusions reached are representative of a rather definite grade of high-class milk production.

The plan of investigation, which is outlined in Table I, provided for the experimental feeding of 12 cows during an entire period of lactation and gestation, which is considered normally to extend over one calendar year; but on some unexplained account the cows did not conceive readily, and three individuals were not pregnant at the time the experiment was

terminated. The slight effect of pregnancy, therefore, in the gross economy of food utilization for milk production, was not present in full normal degree.

Since the main object of the investigation was a study of mineral metabolism, the discussion of the details regarding the experimental program, the animal subjects, and the methods of procedure will be given elsewhere. In brief, however, the conditions of experimentation were as follows:

The subjects were mature Holstein Friesian cows, either high-grade or pure-bred, which were known to be free from tuberculosis and from infectious abortion. As to state of flesh, they were rather uniformly in ordinary working condition at the beginning of the experiment. In the course of the year most of the cows gained in weight about as is usual with cows which are with calf, but No. 1 became sick and lost materially in weight; No. 12 did not quite maintain her weight; while No. 7 improved in condition quite materially.

The experiment began, for each cow, immediately after calving; it was necessary, therefore, to select cows due to calve at about the same time.

The cows received normal rations of hay, grain, and corn silage. Half of the cows received alfalfa hay and half of them timothy hay; and among these two groups two received bone meal, and two received limestone, as mineral supplements, while two received no mineral feeds except for common salt, which all received.

The cows were given approximately as much feed as they would eat without remainder—which signifies that they were virtually full-fed.

On account of the difference in the protein content of timothy and alfalfa hay the grain mixtures fed with the two kinds of hay were compounded in such manner as, in a measure, to compensate for this difference. These grain mixtures were composed of corn meal, ground oats, ground barley, distiller's grains, wheat germ, and soy bean or linseed oil meal. It was not possible, however, exactly to equalize the protein content of the rations, because the relative consumption of grain and roughage was neither constant nor exactly controllable. Also the protein content of the rations lacks critical significance since all of the cows were given more protein than was required—to provide against the possibility that a deficit in protein intake would unfavorably affect the utilization of mineral nutrients.

In the determination of the feeding schedule a prevailing rule of practice was kept in mind, in accord with which a cow is given one per cent of her live weight in hay, three per cent of her live weight in corn silage, and

one pound of grain for each three to three-and-a-half pounds of milk produced; but at no time was it possible to feed all of the cows on exactly this basis, or indeed on any other such schedule of allowance. In fact, in order to maintain the coöperation of the experimental subjects, and to have the rations entirely consumed, it was found necessary to respect the individual preferences and capacities of the cows, and in reality to allow each cow in large measure to determine her own feeding schedule. A continuing effort was made to keep feeding conditions comparable, but it was not possible to accomplish this purpose without compromise.

The obvious preferences of individual cows for particular kinds and quantities of feeding stuffs are not with certainty explicable, but contributing influences seem to be inherited limitations of capacity, the status of the nutritive reserves of the body as determined by previous feeding and production, the condition of the teeth, and the live-weight of the animal in relation to productive capacity.

The hay was fed cut into short lengths, for convenience in weighing, handling, and sampling for chemical analysis; and the grain was all fed ground.

The cows were fed three times per twenty-four hours during liberal milk production, and twice a day later in the period of lactation.

The animals were housed in an ordinary cow shed, with concrete floor and mangers, which was adapted to the experimental purpose by the installation of galvanized iron feed boxes, to prevent waste, and specially designed equipment for the collection of excreta.

In lieu of bedding the cows were provided with thick felt pads, covered with canvas, and nailed to plank overlays on top of the concrete stall floors.

The cows were brushed each day, and scrubbed with soap each week; also they were exercised each day by being led a certain distance—usually about a quarter or half of a mile.

The experimental periods were normally 28 days in length, and energy determinations were made in a bomb calorimeter by standard procedure.

Since there were but two animals on each experimental treatment, the evidence was quite insufficient to warrant the consideration of the effects of the feeding treatment on the economy of utilization of feed energy. It is proper, therefore, only to consider the results as a whole, as indicating in a general way the efficiency of cows to transform feed energy into milk energy (a) during complete periods of lactation, and (b) during a calendar year.

In averaging the data in Table II, cows Nos. 2a and 2b, and 4a and 4b

TABLE I
SCHEDULE OF EXPERIMENTATION

Cow No.	Treatments	Date of beginning	Days on exper.	Days milking dur. exper.	Initial age of cow	Initial functional status of cows	Final functional status of cows
1	Alfalfa hay, grain, silage	Dec. 15, 1929	365	317	4 yrs. 6 mo.	Immed. after calv.	Dry; farrow
2a*	Alfalfa hay, grain, silage	Dec. 16, 1929	123	123	5 yrs. 5 mo.	Immed. after calv.	123 days after calv. 18 days preg.
2b	Alfalfa hay, grain, silage	May 20, 1930	245	182	6 yrs. 9 mo.	120 days after calv.	Dry; 210 days preg.
3	Alfalfa hay, grain, silage, bonemeal	Dec. 10, 1929	292	292	6 yrs. 2 mo.	120 days after calv.	Aborted; 210 days preg.
4a**	Alfalfa hay, grain, silage, bonemeal	Dec. 12, 1929	98	98	7 yrs. 1 mo.	Immed. after calv.	98 days after calv.
4b	Alfalfa hay, grain, silage, bonemeal	May 21, 1930	241	168	5 yrs. 10 mo.	69 days after calv.	Day of calv.
5	Alfalfa hay, grain, silage, limestone	Jan. 11, 1930	365	303	5 yrs. 3 mo.	Immed. after calv.	Dry; 220 days preg.
6	Alfalfa hay, grain, silage, limestone	Dec. 13, 1929	365	317	7 yrs. 11 mo.	Immed. after calv.	Dry; farrow
7	Timothy hay, grain, silage	Dec. 1, 1929	365	310	10 yrs. 11 mo.	Immed. after calv.	Dry; 50 days before calv.
8	Timothy hay, grain, silage	Nov. 30, 1929	365	327	4 yrs. 11 mo.	Immed. after calv.	Dry; 100 days before calv.
9	Timothy hay, grain, silage, bone meal	Dec. 31, 1929	365	304	6 yrs. 9 mo.	Immed. after calv.	Dry; 216 days preg.
10	Timothy hay, grain, silage, bone meal	Dec. 29, 1929	365	305	7 yrs. 10 mo.	Immed. after calv.	Dry; 124 days preg.
11	Timothy hay, grain, silage, limestone	Dec. 20, 1929	365	320	5 yrs. 8 mo.	Dry, 43 days before calv.	Dry; 55 days before calv.
12	Timothy hay, grain, silage, limestone	Dec. 9, 1929	365	319	8 yrs. 9 mo.	Immed. after calv.	Dry; farrow

* Removed from the experiment because of a tumor in the nasal passage.

** Removed from the experiment because of an abscess on the heart, caused by penetration by a foreign body in the rumen.

TABLE II

THE EFFICIENCY OF TRANSFORMATION OF FEED ENERGY INTO MILK ENERGY

Cow No.	Treatment	Days of lactation	Milk produced	Gross energy of feed during lactation	Energy of milk	Recovery of feed energy during lactation	Gross energy of feed dur-during 365 days	Energy of milk divided by energy of feed for 365 days	Initial live weight	Final live weight (365 days)
			Kgm.	Cals.	Cals.	Per cent	Cals.	Per cent	Kgm.	Kgm.
1	Alfalfa hay, grain, silage	317	6,762.3	20,506,160	4,400,238	21.46	22,688,490	19.39	661	611
2a; 2b	Alfalfa hay, grain, silage	305*	4,422.6	15,728,240	3,084,259	19.61	18,418,965	16.75	489; 513	—
3	Alfalfa hay, grain, silage, bonemeal	292	4,808.7	15,820,334	3,147,747	19.90			579	—
4a; 4b	Alfalfa hay, grain, silage, bonemeal	266**	4,036.1	14,201,883	2,917,072	20.54			602; 456	—
5	Alfalfa hay, grain, silage, limestone	303	5,200.4	16,278,297	3,403,506	20.91	18,933,379	17.98	528	620
6	Alfalfa hay, grain, silage, limestone	317	5,847.1	18,007,650	3,954,107	21.96	19,826,079	19.94	526	564
7	Timothy hay, grain, silage	310	5,303.1	17,553,839	3,167,222	18.04	20,178,974	15.70	559	714
8	Timothy hay, grain, silage	327	5,438.4	18,541,678	3,511,764	18.94	20,087,435	17.48	497	590
9	Timothy hay, grain, silage, bonemeal	304	5,332.7	15,970,094	3,524,987	22.07	18,315,622	19.25	478	547
10	Timothy hay, grain, silage, bonemeal	305	4,208.8	14,421,704	2,963,628	20.55	16,571,279	17.88	435	497
11	Timothy hay, grain, silage, limestone	320	5,730.2	17,635,042	3,853,669	21.85	19,530,045	19.73	493	522
12	Timothy hay, grain, silage, limestone	319	4,380.2	14,022,000	3,273,667	23.35	15,503,340	21.12	425	415

* Cow 2a, 123 days, and cow 2b, 182 days.

** Cow 4a, 98 days, and cow 4b, 168 days.

were eliminated because in these cases two cows were used to cover the year; and No. 3 was eliminated because she did not finish the calendar year of experimentation. The results as determined for these cows, however, are not without value.

The averages of the data for the remaining nine cows are as follows: Live weight of cows, 520.8 kgm. (1146 lbs.); milk produced per year of lactation, 5,356 kgm. (11,783 lbs.); recovery of feed energy during lactation, 20.96 per cent; and recovery of feed energy during a calendar year, 18.68 per cent.

These rates of efficiency were obviously related to gain or loss of body weight. Thus, the three farrow cows, Nos. 1, 6 and 12, were characterized by relatively high rates of efficiency in transforming feed energy into milk energy; but the nearly equal performance of three other cows, Nos. 5, 9, and 11, which at the end of the year were advanced in pregnancy, indicates that under the existing conditions the energy requirements of gestation did not constitute a major influence as affecting efficiency of milk production.

The arrangement of the cows in the order of their gain in weight, however, incidentally arranges them, in general, in the reverse order of their efficiency to transform feed energy into milk energy. This generalization is most notably untrue in relation to one cow, No. 1, which was the best producer of all. This cow appears not to have been as efficient as implied by her failure to gain in weight, this departure from the general order being due, at least in part, to the fact that she became sick, and lost markedly in live weight during her twelfth month on experiment, apparently as a result of the death of her fetus about two-and-a-half months after conception; also this cow occasionally leaked milk from the udder, which was not accounted for, her true efficiency as a milk producer, therefore, being somewhat greater than as indicated by the quantity of milk with which she was credited.

The most efficient cow, No. 12, did not conceive during the experiment; she did not quite maintain her live weight; and she transformed 23.3 per cent of her feed energy into milk energy during 319 days of lactation (21.1 per cent during 365 days).

The least efficient cow, No. 7, gained 155 kgm. in live weight, produced a calf, and transformed 18.04 per cent of her food energy into milk energy during her 310 days of lactation (15.7 per cent during 365 days).

Summary

Nine Holstein Friesian cows, with an average live weight of 520.8 kgm. (1146 lbs.), and an average milk production, for one period of lactation,

of 5.356 kgm. (11,783 lbs.), transformed 20.96 per cent of their feed energy into milk energy during a period of lactation averaging 313 days in length; and transformed 18.68 per cent of their feed energy into milk energy during a calendar year.

The most efficient cow converted 23.35 per cent of her feed energy, and the least efficient cow 18.04 per cent of her feed energy, into milk energy during the period of lactation.

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A COMPARATIVE STUDY OF RABBITS MAINTAINED ON BARLEY OR ALFALFA

By

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INTRODUCTION

IN EARLY diet experiments from this laboratory concerned with the production of renal and blood vessel changes, Nuzum, Osborne, and Sansum (1) concluded that "there also was evidence of acidosis, as shown by a continued decrease in the carbon dioxide of the blood plasma of the oat and liver protein groups of animals." In later experiments (2), the same liver diet (urine pH 6.2) for the same period was fed rabbits with spontaneous nephritis and the results compared with rabbits with spontaneous nephritis on a barley-alfalfa diet (urine pH 7.0 to 8.5). In spite of the kidney damage, the rabbits on the liver diet failed to show evidence of acidosis as judged by a comparison of the CO_2 combining powers of the series on liver with the series on barley and alfalfa. The initial mean for the CO_2 combining powers was 47.6 with a standard deviation of 2.7, eight samples being taken. At the termination of the diet régime, the mean for each series, with four in a series, was well within the standard deviation of the mean. Judging by the standard deviation of the mean established by the figures of Nuzum's latter series, the normal variation for his controls in the earlier paper was five times the standard deviation of the mean. Page (3) compared rabbits on an acid diet of oats and bread with rabbits on an alkaline diet consisting of cabbage, carrots and hay. The alkali reserve was determined weekly for a period of five weeks. The results for the acid diet are within once the standard deviation of the mean of the initial values of Nuzum's second series. The alkali reserve for the alkaline diet series was considerably higher even after one week of dieting. However, these rabbits developed a diarrhea and showed loss in weight in a week's time, so that the condition of alkalosis can hardly be ascribed to the alkaline ash of the diet. A similar experience in a human experiment of our series will be described later in another paper. The remarkable agreement between Nuzum's second initial series and Page's values after an acid diet is evidence against the production of a lowered alkali reserve in the rabbit by an acid ash diet.

The earlier work of Nuzum, Sansum, and Osborne indicated a production of acidosis in the rabbit by diet and suggested a possible change in the pH of the blood. The original purpose of the present experiments was to establish whether or not an acid diet was able to change the pH of the blood, using the newer refined methods of analysis.

Experimental

Diet régime. Fourteen rabbits approximately six months of age were divided into two groups. In each group three rabbits were selected for the successive determinations of blood pH and CO_2 content, the blood to be drawn from the heart. The remaining rabbits were to be kept for substitution in case of accidental death due to injury to the heart, or, barring this accident, to serve as controls for the effect of continued heart puncture throughout the experiment. For two weeks both groups were placed on a mixed alfalfa-barley diet, the blood data being determined for the three selected rabbits in each group. The one group was then placed exclusively on alfalfa and the other group exclusively on barley for the next six months. During this period it was observed that the rabbits on the barley diet destroyed the wooden slats of their cages, making an attempt to eat the wood. New cages lined with metal were installed when this observation was made. Eating the wooden slats had no effect upon the acidity of the urine, so would not be a factor in the experiment. At any rate, the time interval during which the wood was eaten was a small fraction of the total period of the experiment. As originally planned, the experiment was to be terminated at this time, but, as no chemical changes in the blood could be detected, and as the rabbits had all survived, the regime was continued until all the animals on the barley diet were dead. From the sixth month on, the rabbits on the barley diet were given a small quantity (20 gm.) of alfalfa once a week. Food was withheld the night before the morning when the bleedings were made. The rabbits lived outdoors throughout the experiment, except when urine collections were made. The rabbits were then placed in metabolism cages and fed their barley or alfalfa *ad libitum*, a twenty-four hour specimen being collected. Two of the rabbits on the alfalfa diet were killed at the time the first rabbits on the barley diet died. The remainder of the alfalfa group was sacrificed two months after all the rabbits on the barley diet had died.

Methods. The samples of blood for pH and total CO_2 determination are valueless unless taken under basal conditions. In working with laboratory animals, this presents a problem of considerable difficulty and one which has in the past been entirely overlooked by many workers, so that other-

wise excellent results are worthless for interpretation. In the earlier work in this laboratory, blood for CO_2 combining power was obtained by cutting the marginal ear vein of the rabbit and collecting the blood under oil. In order to facilitate the speed of bleeding, the blood vessels of the ear were either dilated by rubbing the ear with ether or by placing the ear over an electric light bulb. There are two serious objections to this procedure; (1) an unavoidable loss of CO_2 due to the large volume of oil required and the long time required for bleeding, and (2) the uncertainty as to whether the blood was venous or arterial, since venous blood becomes virtually arterial with marked dilation of the vessels. To establish the possible error of this procedure samples of venous and arterial blood were taken from a rabbit's heart, a procedure which was successful after some practice, and both the pH and total CO_2 content determined. The pH of the venous blood was 7.46, that of the arterial, 7.49. The total (CO_2) of the venous blood plasma was 51.9 volumes per cent, that of the arterial blood 39.0 volumes per cent. The great variation in the CO_2 combining powers of the control groups in Nuzum's first series, as compared with the second series, may possibly be accounted for by the early technic of blood sampling. The practice of stunning rats by a blow on the head and then bleeding from the heart is a common practice in some laboratories and gives erratic results due to the changes of respiration, leading to high or low results depending upon over- or under-ventilation. An example is cited. A rat was bled from the heart. He was then stunned by a blow on the head and again bled from the heart. The first plasma pH was 7.53, the second, 7.16. In the present studies the blood was drawn from the heart.

The pH of the urine of the rabbits on the barley diet was determined by means of the quinhydrone electrode. The urine of the rabbits on the alfalfa diet was too alkaline for the range of this electrode. The pH was determined colorimetrically with thymol blue. No degree of accuracy can be claimed for the alkaline urines, because of the heavy precipitate which they always contain. If the precipitate be removed by centrifugation, the pH of the supernatant liquid on dilution will not be the same as when the solids are present. After the first seven months of the experiment, the urines were examined for albumin and casts.

The plasma pH was determined on the plasma as drawn, as described in earlier publications (4). The total CO_2 content was determined in the same sample.

Results

Blood amino-acid and urea nitrogen. Data for blood urea and amino-acid nitrogen were obtained for five of the rabbits on the acid diet just

before death. Both increases in the blood amino-acid nitrogen and liver changes as indicated by histologic examination were observed in two of the acid diet rabbits. The blood amino-acid nitrogens for the three other rabbits on the acid diet and those for three rabbits on the alkaline diet, determined at the same time as control values, were within the normal range. All the urea nitrogen values were within the normal range with the exception of that for the rabbit which had spontaneous focal nephritis. Data for this rabbit were obtained the day preceding and the day of death. It is interesting that the day preceding death both values are within normal limits, the very rapid rise (urea from 38.5 to 89.3, amino-acid nitrogen from 9.4 to 18.2) occurring in less than twenty-four hours. No significance can be attached to this isolated observation.

Weights of liver and kidneys. Since the rabbits on the barley diet died at intervals over a considerable period of time, there is no basis for comparison of the weights of the organs with the organs of rabbits on the alfalfa diet at the corresponding time. The weights were nevertheless recorded. The average for both kidneys for the rabbits on the barley diet was 14.1 gm., for the rabbits on the alfalfa diet, 20.2 gm. The average body weight for the former was 2.6 kilos., for the latter, 3.7. The size of the kidneys followed roughly the body weight. For the liver, the converse was true, the livers of the rabbits on the barley diet averaging 84.8 gm., those of the rabbits on the alfalfa diet, 72.0 gm.

Urine pH. The pH of a 24-hour urine specimen was determined each time the blood pH was taken. For the rabbits on the barley diet, the range of urine pH was 5.2 to 6.2. For the rabbits on the alfalfa diet, the range was 8.2 to 9.6. The individual values are not reported since they are of no significance. None of the urines of the alkaline series showed either albumin or casts at any time of the experiment. The urines of the acid series invariably gave slight positive tests for albumin. Casts were rarely found. The individual findings are not reported since there were no exceptions to the generalization.

Plasma pH. Of five rabbits on the acid barley diet[1] from 16 to 23 months,

[1] Another series of ten rabbits was given exclusively unpolished rice. The range of urinary acidity was pH 5.0 to 6.0. After five months, evidence of vitamin deficiency was marked, and after ten months six of the rabbits had died. Plasma pH and total CO_2 were determined five months after the rice diet was begun.

Plasma pH: 7.48, 7.41, 7.40, 7.42, 7.47, 7.45, 7.43. Mean 7.44 ± 0.01.

Plasma total CO_2: 46.4, 39.0, 50.0, 36.9, 44.9, 46.1, 50.1. Mean 44.8 ± 2.2.

The values show no significant deviation from the initial control values. While the time interval for the rice series is much shorter than for the barley series, the inferences to be drawn from the experiments are the same; during a period of gross dietary abuse carried to the point of death, the acid base equilibrium of the blood remained unchanged on a diet high in acid ash.

only one showed evidence of a terminal acidosis. Histologically there was a focal spontaneous nephritis. Even including the data for this rabbit, the mean for the five terminal total plasma CO_2 values for the acid diet rabbits is 49.2 ± 4.2 volumes per cent, while that for the value for six rabbits at the beginning of the experiment was 47.2 ± 3.6 volumes per cent. Likewise, the pH values, including the rabbit with the kidney damage, show no change, the mean for the terminal acid diet rabbits being 7.44 ± 0.05, that for six rabbits at the beginning of the experiment, 7.45 ± 0.02. In examining the total plasma CO_2 and pH data for the rabbit, one notes a large normal variation for the same rabbit from day to day, with an occasional erratic result. It is possible that the occasional erratic result obtained was due to temporary injury to the heart by puncture. The final results, both chemical and histologic, indicated no permanent injury. An example is Rabbit 6, on the alfalfa diet. The total CO_2 during two years fluctuated between 39 and 46 volumes per cent with one low value of 25.0 per cent. In human beings such a variation is not encountered, there apparently being a more delicately balanced mechanism for maintaining a narrow range. The normal plasma pH range for human venous blood determined by us with the quinhydrone electrode is 7.40 to 7.50. From data taken from fifteen different subjects, Cullen and Earle (5) obtained exactly the same range. Our values for the arterial blood of the rabbit cover the same range overlapping both extremes by 0.05 pH.

TABLE I

BLOOD UREA AND AMINO-ACID NITROGEN VALUES FOR BARLEY SERIES PRIOR TO DEATH WITH CONTROL VALUES FROM ALFALFA SERIES.

Rabbit	Date	Urea N.	Amino-acid N.
201, Barley	June 11, 1930	38.5	9.4
	June 12, 1930	89.3	18.2
203, Barley	June 11, 1930	19.6	—
210, Barley	Aug. 6, 1930	33.3	15.5
202, Barley	Jan. 7, 1931	12.9	10.2
212, Barley	Jan. 7, 1931	25.0	11.6
204, Alfalfa	June 12, 1930	19.8	9.4
205, Alfalfa	June 12, 1930	27.8	9.3
206, Alfalfa	June 12, 1930	30.3	9.7

The total plasma CO_2 of the rabbits maintained on alfalfa was not significantly changed at the end of two years. The mean for five rabbits was 41.9 ± 2.5 volumes per cent, which is lower by a little over once the standard deviation of the mean than the initial control value of 47.2 ± 3.6 volumes per cent. Likewise, the plasma pH values show no change, the mean for the six terminal values being 7.46 ± 0.02.

TABLE II

ACID BASE DATA AND HISTOLOGY OF RABBITS ON PROLONGED BARLEY OR ALFALFA DIET

Diet and Rabbit No.	pH and Total Plasma CO_2 of Blood											Histology			
	Feb. 1929	March* 1929	Apr. 1929	May 1929	Sept. 1929	Dec. 1929	March 1930	June 1930	Aug. 1930	Jan. 1931	March 1931	Kidney	Liver	Aorta	Heart Coronary
Barley 1	7.46	7.46 46.0	7.50 40.2	7.50 47.3	7.36 35.5	7.43 37.0	7.42 34.0	7.27D 35.0				Spon. focal nephritis	Cirrhosis	—	—
2	7.48	7.48 50.6	7.54 46.7	7.44 46.4	7.49 47.9	7.48 45.6	7.44 33.6			7.44D 56.7		—	Slight fatty change	—	—
3	7.51											—	—	—	—
4		7.43 49.4	7.49 52.6	7.53 57.7	7.45 47.0	7.45 41.8	7.48 40.1	7.58K 50.1				—	—	—	—
10									7.48D 58.7			—	Moderate fatty change	—	—
12										7.44K 45.8		—	—	—	—
11							D								

* Average of 4 determinations made weekly.

D—Died. K—Killed.

TABLE II (*Continued*).

Diet and Rabbit No.	pH and Total Plasma CO_2 of Blood											Histology			
	Feb. 1929	March* 1929	Apr. 1929	May 1929	Sept. 1929	Dec. 1929	March 1930	June 1930	Aug. 1930	Jan. 1931	March 1931	Kidney	Liver	Aorta	Heart Coronary
Alfalfa 4	7.36 32.8	7.44 43.9	7.48 43.7		7.52 49.8		7.45 48.4	7.48 43.7			7.45K 39.7	—	—	—	—
5	7.43 49.7	7.42 41.5		7.44 46.7	7.45 40.4	7.47 41.7	7.47 43.6	7.45 39.5			7.45K 39.5	—	—	—	—
6	7.44 46.2	7.44 41.6	7.40 25.2	7.42 40.3	7.47 40.2	7.41 40.4	7.46 41.5	7.47 44.2			7.47K 39.0	—	—	—	—
3											7.49K 51.8	—	—	—	—
9									7.39K			—	—	—	—
30									7.52K 39.6			—	—	—	—
7										D		—	—	—	—

* Average of 4 determinations made weekly.
D—Died. K—Killed.

Histology

Autopsies were made within a few hours after death and tissues from the kidney, liver, aorta and heart were fixed in Zenker's solution, embedded in paraffin, and stained with hematoxylin and eosin. The results of the histologic examinations are briefly recorded in Table II, the minus sign indicating that no change is noted. In sections of the kidneys of Rabbit 1, on the barley diet, there is the usual picture of a slight degree of spontaneous focal nephritis. There are dilated tubules with flattened or desquamated lining cells, focal regions of small, round cell infiltration in which the tubules are compressed and atrophic, and unchanged glomeruli and blood vessels. This condition has been carefully described by Bell and Hartzell (6), by Leiter (7), by Le Count and Jackson (8), and others.

In the livers of Rabbits 2 and 10 there are slight and moderate degrees of fatty change, respectively. In the higher grade of involvement, apparently not more than 20 per cent of the hepatic parenchyma is affected. The spontaneous occurrence of the liver changes described is sufficient to account for their presence, and so no causal relationship can be attributed to the diet.

Discussion

The results of the present study show that the rabbit is not as sensitive to changes in acid ash or alkaline ash intake as has been supposed. On the alkaline side he is better able to cope with alkali excess than is the human being, a constant passage of a urine in the range pH 8.2 to 9.5 having no effect upon the plasma pH or CO_2 content. The range of urine acidity on the barley diet, pH 5.2–6.2, is one which produces no shift in the acid base equilibria in the blood of humans. Whether the rabbit is more sensitive to excess acid than the human can not be decided from these studies. Since rabbits on a prolonged liver or oats diet develop degenerative blood vessel changes, while those on a barley diet do not, the range of urinary acidity for all three diets being the same, one is forced to ascribe the changes produced by the liver and oats diet to some factor other than the acid ash. If the barley diet, which was obviously deficient, had produced such changes, the proof would still be lacking that the effects were not secondary, due to some such factor as vitamin deficiency or infection. The experiments with barley show rather dramatically that in spite of gross dietary abuse carried to the point of death, the acid base equilibrium remained normal to the end.

Summary

There was no significant change in the plasma pH or CO_2 content of the blood of rabbits maintained on a barley diet for a period of nearly

two years, as compared with rabbits on an alfalfa diet, the urinary acidity of the barley group ranging from pH 5.2 to 6.2, that of the alfalfa group from pH 8.2 to 9.5.

No pathological changes were noted for the seven rabbits maintained on alfalfa. Early death (16 to 23 months survival) with clear cut evidence of diet deficiency was noted for the barley diet, fatty change of the liver being present in three of six rabbits and a focal diffuse nephritis in one.

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ACTION OF RADIO-ACTIVE SUBSTANCES ON VITAMINS*

By

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THE Department of Chemistry frequently has in its possession preparations of radio-active materials, so it seems desirable to take the opportunity of studying their action on vitamins. A number of workers have studied the action of rays of short wave length on these substances, and it is clear that at least one of them, vitamin A, is quite labile.

Zilva (1) exposed milk fat to the rays from a quartz mercury arc, and observed that it was bleached, acquired the odor of tallow, and became unpalatable. Vitamin A was destroyed. In a later study (2) he concluded the destruction of the vitamin was due to ozone, for if the exposure were conducted in an atmosphere of carbon dioxide there was no loss of vitamin activity. Spinka (3) subjected milk to a similar procedure, and concluded that toxic substances developed which killed the experimental animals, but vitamin A was not destroyed. Zilva (4) remained convinced, however, that in his experiments destruction of the vitamin had occurred. Titus, Hughes, Hinshaw, and Fitch (5) exposed milk to the rays of a quartz-mercury arc, and observed destruction of vitamin A. Supplee and Dow (6) irradiated milk for very short periods, a few seconds up to 3 ½ minutes, but were not able to detect any destruction of the vitamin. Steenbock and Coward (7) regarded vitamin A as remarkably stable to ultra-violet rays. The effect of rays of still greater frequency has apparently received little attention.

As to the effect of rays of short wave length on the water-soluble vitamins there is at present no unanimity of opinion. Hogan and Hunter (8) reviewed the earlier literature on this topic, and reported that one of the factors in the vitamin B complex, stable to heat, is destroyed by ultra-violet irradiation. No attempt was made to determine whether the destruction was complete, or whether partial destruction of other factors occurred. Chick and Roscoe (9) confirmed this report in its essentials, and

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agreed that one factor, G (B_2 in their terminology) undergoes very considerable destruction. In addition they observed that B(B_1) is also partly destroyed. An attempt (10) was made in another department of this institution to apply the technic of Hogan and Hunter to a study of the distribution of vitamins B and G in certain foodstuffs. The behavior of both control and experimental animals indicated that the method could be used successfully, but after several months the observations were interrupted. On resuming these studies, all attempts to repeat the previous work apparently met complete failure. The only alteration in the experimental conditions mentioned was a change in the yeast supply. Palmer and Kennedy (11) were unable to detect any destruction of either factor. Guerrant and Salmon (12) estimated from their experience that approximately 25 per cent of vitamin G is destroyed by irradiation, and evidently additional study will be required to explain the discrepancies. Hottinger (13) reported that the vitamin C content of whole dried milk was not reduced by ultra-violet radiation.

It was established by Steenbock (14) and Hess (15) some time ago that foodstuffs acquire antirachitic potency on irradiation with ultra-violet rays. There is still some dispute as to the possible precursors of vitamin D, but the concensus of opinion supports the view of Rosenheim and Webster (16) that ergosterol is the parent substance. According to Knudson (17) ergosterol may also be activated by cathode rays.

Morrison, Peacock, and Wright (18) state that x-rays have a destructive effect on vitamin D.

Experimental

Both radiothorium and mesothorium were available at various times, and they were used interchangeably, depending on which was available at the time. They were in the form of chlorides or bromides and, though highly concentrated, they were far from being chemically pure. The radioactive materials were loosely cemented to the inside of small containers, either crucibles or flat crystallizing dishes, and covered with mica to prevent loss of the active substance. This prevented bombardment by alpha particles, and reduced in some degree the intensity of the beta-rays. The activity of the various preparations, measured by the gamma-ray method, varied between 20 and 25 milligrams of radium.

Albino rats, reared on Steenbock's (19) ration, were used as experimental animals. In some cases these were transferred to the vitamin-A-free diet at 19 days of age, a procedure suggested by Nelson (20). In our experience this modification greatly reduces the variability among test

animals. The length of the depletion periods becomes more uniform, and practically all individuals develop xerophthalmia within a few days of each other. The composition of one of our experimental diets, No. 925, is given below.

	Per Cent
Egg white	20
Dextrin	64
Dried yeast	9
Mineral salts	4
Agar	2
Irradiated cotton seed oil	1

The composition of No. 924 was the same, except that yeast was eliminated and the dextrin increased to 73 per cent. Yeast was supplied separately, 500 mgm. daily.

Our first observations were made on vitamin A, and butter was the chief source of material. It was placed in a low crystallizing dish, 9 cm. in diameter, in a layer about 0.8 cm. deep. The radio-active substance was placed directly over this, with the mica about 2.5 cm. above the butter, and allowed to stand for 96 hours. After 72 hours a thin area immediately beneath the container was distinctly bleached, and 24 hours later it was practically colorless. The butter was then transferred to a closed glass container and stored in the refrigerator. The bleaching process continued, however, for when examined later the entire sample was colorless.

The rats used for the vitamin tests were approximately 23 days old when selected and weighed about 40 grams. They were placed on Ration 925, free from vitamin A, for a depletion period, usually about 5 weeks. The experimental period was begun when the animals began to lose weight, though usually some ophthalmias had also appeared. The animals were then divided into three groups, the first of which was continued on Ration 925. The purpose of this group was to determine the survival period of rats on a ration that is practically free of vitamin A. The other animals were changed to Ration 924, deficient in both vitamins A and B. It was anticipated that the exposed butter might be unpalatable, and so would not be consumed readily. It was hoped that if the vitamin B supplement, dried yeast, were mixed with the butter, complete consumption would be secured. This expedient may have been unnecessary, but at any rate there were no difficulties because of failure to consume the supplements. These were weighed out daily into small glass containers. Group II received treated butter, and Group III received the untreated material. Both

samples came from the same package, and were stored under the same conditions. Each rat in a group had a litter mate in the others, of the same sex and of approximately the same weight.

It is evident from Fig. 1 that the exposed butter gave no evidence of vitamin A activity, as judged by rate of growth, incidence of ophthalmia, or survival period. The behavior of the animals was practically identical with that of the negative controls. The positive controls were either cured of ophthalmia, or protected.

Cod liver oil was also exposed to radiothorium, and though no change in the oil was visible, it apparently lost its vitamin A potency. Beginning on the 60th day, Rat 2824 (Fig. 1) was given a daily dose of 14 mgm. of the exposed oil, without affecting in the least the course of the disease. During the last 8 days shown on the graph, Rat 2833 was given similar treatment, with the same result. Numerous trials, not recorded here, have shown that this amount of the untreated cod liver oil is more than sufficient to meet the vitamin A requirement of the rat.

As previously mentioned, the bleaching process in butter continued after removal from the vicinity of the radio-active substance. This suggested that the destruction of the vitamin may not be due directly to exposure to the radio-active material, but is an indirect effect. This may be explained as due to an acceleration of the changes that normally occur, or to the formation of some unusual substance which has a destructive action. It was decided, therefore, to mix an exposed, non-potent sample of butter with an equal weight of untreated material, and test the potency of the mixture after standing for various periods of time. A portion of the untreated butter was also set aside for comparison.

The procedure used in making the tests was varied a little from that previously described. At the end of the depletion period, all rats were transferred to Ration 924. Each received the same amount of dried yeast, and of the fat supplement. For Group I this was 200 mgm. of hydrogenated vegetable oil.[1] Group II received 200 mgm. of a mixture of equal parts of exposed and untreated butter. Group III received a mixture of equal parts untreated butter and the vegetable oil. In the earlier trials no diminution of vitamin A activity was detected, and these rats are not shown in Fig. 1. In the first trial the mixture was prepared the same day the test feeding was begun, and continued for 28 days, without obtaining any evidence of vitamin A insufficiency. In the meantime the negative controls had developed severe ophthalmia and died. All butter supplements were then

[1] Crisco.

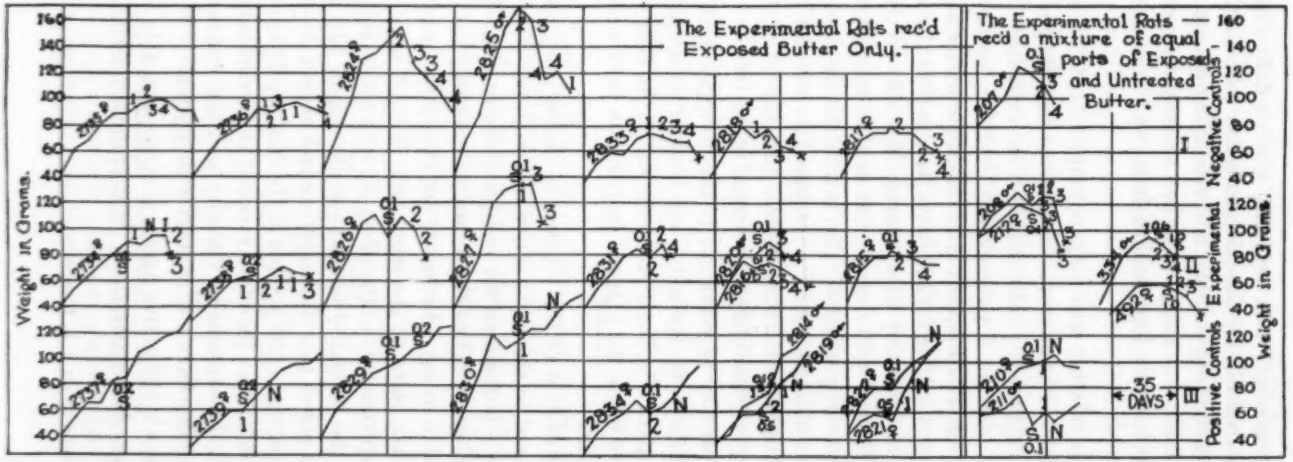

FIG. 1. Rats in the upper row received no vitamin A supplement. All experimental rats received butter that had been exposed to radio-active substances. Of these animals, Nos. 208, 212, 334, and 492 received a mixture which contained, in addition to the treated material, an equal amount of untreated butter. The mixture had been stored 200 days when first used. The animals in the lower row received untreated butter.

The point at which the supplements were first fed is indicated by the letter S. The amount of the supplement is indicated in grams just above, or below the letter. The severity of the ophthalmias is indicated by the numerals, 1, 2, 3, and 4. If an animal recovered from ophthalmia the fact is indicated by the letter N. Deaths are indicated by the mark, x.

discontinued, and the remaining 5 rats were each given a daily dose of 14 mgm. of the treated cod liver oil. By the thirteenth day following, one of them had developed a marked ophthalmia, and was losing weight. It was then given the untreated oil, and 7 days later had entirely recovered. By this time the other 4 rats had developed ophthalmias and were also losing weight.

In the second trial the mixture was prepared April 22, 1929, and the test feeding began August 2. The mixture was almost completely bleached within 8 weeks, and was apparently colorless when the experiment began. Again no destruction of vitamin A potency was detected, though slight loss may have occurred. In a subsequent trial this same material, mixed on April 22, was used after standing for 200 days. By this time the vitamin activity of the sample had entirely disappeared. Rat 334 received 0.6 grams daily, and Rat 492 received 1.0 gram, but neither ophthalmias nor survial periods were apparently affected. This is a relatively slow rate of destruction, and it seems unlikely that the disappearance of the vitamin is the only change that occurs. One may assume that the vitamin is destroyed by intermediate compounds that are formed in the fat.

Another observation worthy of comment is the disappearance of the vitamin A color test as given by the antimony trichloride reagent (21). Untreated cod liver oil gives a brilliant reaction, but at the time the biological examinations were made the exposed preparation gave a negative test[2] with the color reagent.

It is evident that destruction of the vitamin in butter proceeds much more rapidly while exposed to the radio-active material, but our data do not show how much time is required for complete destruction. The exposures were made some weeks before the animals were ready for the test feeding, and we are unable to say whether or not the losses in color and in vitamin A potency take place at the same rate. We will also reserve, until a later date, any expression as to the effect of radio-active substances on cod liver oil. When the biological test was made the oil had become quite viscous and it was noted that the experimental sample occupied a considerably lower percentage of the volume of the container than did the control. The smaller sample was therefore exposed to a relatively larger volume of air, and both the increase in viscosity and the destruction of vitamin A may have been due merely to contact with oxygen.

As to which of the rays is the active agent, it seems probable this is a property of the beta-ray. All preparations were protected behind mica,

[2] These observations were made by Dr. J. E. Hunter.

which shields out the alpha-particles. Milk fat was later covered with a lead plate, then exposed to mesothorium. This would shield out all but a small part, possibly 5 per cent, of the entire beta-ray activity, but would reduce the gamma-ray activity scarcely at all. The milk fat exposed in this manner was not perceptibly bleached, and no decrease of vitamin A activity was detected. It is worthy of note that Hanson and Heys (22) found that the lethal mutations produced by exposing Drosophila to the rays of radium are due entirely to the beta and not at all to gamma rays. It is possible, however, that the two phenomena, bleaching, and loss of vitamin A potency, are more or less independent.

In addition, observations were also made to determine whether vitamins B, C, D, and G are labile when exposed to radio-active substances. The Osborne-Wakeman (23) concentrate was used as a source of both B and G. Concentrated orange juice[3] was used as a source of vitamin C. Both cod liver oil, and a commercial preparation of activated ergosterol,[4] were used as sources of vitamin D. No destruction was detected in any case. Ergosterol was readily activated by ultra-violet rays so an effort was made to activate it by exposure, under similar conditions, to mesothorium. Some of the exposures were for 6 hours, some for 96, but no antirachitic potency could be detected. In as much as the results were negative, the procedures are not described in detail.

Summary

1. Vitamin A of milk fat is destroyed by exposure to radio-active substances. The destruction may be indirect.

2. The beta-ray is apparently the active agent.

3. The evidence indicates that vitamins B, C, D, and G are stable when exposed to beta or gamma rays.

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[3] Supplied by the California Orange Grower's Association.

[4] Acterol. We wish to express our appreciation of the kindness of Dr. C. E. Bills of Mead Johnson and Co., who supplied the acterol and ergosterol, and gave valuable advice as to methods of activating ergosterol with ultra-violet rays.

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SALINE AND ALKALINE DRINKING WATERS

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A NUMBER of years ago a preliminary report (1) was made of the effect of saline drinking waters upon growth and reproduction of animals. Since that time the problem has been continued and has increased in interest as well as in scope and practical application. As stated in the previous article, of the several nutriments needed by man and animals, drinking water is undoubtedly the greatest variable in this day of rapid travel and the one most difficult of correction in many regions of the United States. There are large areas of the earth's surface where deep wells cannot be used due to the extreme alkalinity or salinity of waters procured. Many streams flow through salt beds on the surface or through areas where they become contaminated with the waters from oil wells, factories or mines. When the flow of water is limited, due to dry seasons, the dissolved salts make the use of these waters questionable. In other sections alkali beds of great area become leached by rains or underground waters, causing the water to be even more deleterious for birds or aquatic life.

A search of the literature, as previously stated, reveals little to answer the question of a possible standard for drinking purposes and the physiological effects of an excess of salts. The U. S. Public Health Service (2) has set up an arbitrary standard for interstate carriers, which may be an ideal to be sought but one not possible to obtain in many regions. The pharmacologic literature is very complete in regard to the effect of minimum lethal doses of specific salts but is of little use in postulating the effect of a continued use of these salts in drinking waters. Volumes have been written by developers of mineral springs lauding their merits, especially in the literature of a generation ago; but as stated by Van Noorden (3), "The truth is that so little is known of the bearing of mineral waters on biological processes that most of the statements in biological literature may be stigmatized as idle make-believe." There are many articles having some bearing on the problem, but they are of no direct importance in the solution of our difficulty. In the medical literature we find references to the effect of certain waters in the stimulating of intestinal secretions, their effect upon digestion, and upon hydrogen ion concentration of the body fluids. Chase (4) makes a report of the apparent health of people living in

hard water sections of Ohio compared to the health of those living in soft water regions. Roberts (5) states that young fish can stand Cl up to 5,750 parts per million; while Young (6) believes that a shift in the osmotic effect to about six atmospheres, due to total salt, is fatal. Shaw (7) in his duck disease studies of salt areas makes record of the amount of soluble salts found necessary to injure and kill migrating ducks.

A few articles having more direct bearing upon the subject have appeared in the literature since the publication of our first article. These pertain to deaths thought to have been caused by saline waters in the British Colonies. Legg (8) describes the death of sheep that accidentally drank salt solutions while being shipped. Ramsay (9) states that cattle in cases can live on water containing 17,120 p.p.m. of total salt and thrive on 11,400 p.p.m. Horses thrive on waters containing up to 5,720 p.p.m. and can be sustained on 9,140 p.p.m., if not worked too hard; while sheep are similar to cattle in this respect. Scott (10) gives a rather detailed description of the clinical symptoms of cattle suffering from the effects of salty waters.

Clough (11) and Mitchell, Carl and Carman (12) refer to deaths of poultry from the excessive consumption of ordinary table salt. We have observed in many cases deaths among hogs and cattle that had been overfed fine salt while in a salt-hungry condition. Hogs, especially, seem to be particularly susceptible. In most of these cases, however, we are dealing with the effect of single large doses of salts in salt solution or with unknown mixtures. In our problem it is necessary to know the effect of continuous use of such solutions over long periods of time. Likewise, it is necessary to know whether there is possibly some physiological change whereby the body can adjust itself to these altered conditions. Such a change has been noted (13) in freezing points of blood of eels after immersing them in saline water, indicating that some salts are evidently absorbed and retained in the blood. The writer has often found both man and animal sustaining no apparent injury from continuously using waters that caused very serious discomfort and in some cases death to animals not accustomed to such concentrations.

The purpose of this investigation has been to demonstrate what concentrations of various commonly occurring salts and alkalies produced death, stunted growth, inhibited reproduction and disturbed lactation; to determine by mixture of salts if there is an antagonistic reaction between salt ions as has been proved to be effective for plants; to ascertain if there is some physiological readjustment by which animals can become accustomed to saline drinking waters; and lastly, to make a study of the dele-

terious condition produced and mechanisms by which physiological conditions are altered.

Method of Procedure

In all related studies undertaken by other workers, it has been the custom when deaths occurred to obtain a sample of the water which produced the effect and to make an analysis of it. In these cases it was then necessary to postulate what was the effect of the mixture. It has seemed advisable in our case to proceed by a method previously used in plant nutrition studies, that is, to use distilled water and add varying amounts of the salt under consideration until the deleterious levels were determined. Rats were used as experimental animals for all preliminary tests, because of convenience of control, the possibility of using very large numbers, and comparatively short reproductive periods. The animals were housed in cylindrical wire cages commonly used for nutrition tests, and fed a well-balanced ration (consisting of yellow corn, whole wheat, milk powder, alfalfa, cottonseed meal, and tankage) regularly used for our breeding colony. One per cent of sodium chloride and 1 per cent of calcium carbonate were added for mineral supplements. The drinking water was furnished from glass bottles fitted with glass syphons. The usual precautions in regard to measuring the amount of water and feed consumed, the recording of weights of all animals, and the practice of sanitary measures familiar to nutrition workers were observed.

The first studies were made with sodium chloride, that being the most widely distributed salt in most regions. Ten solutions containing sodium chloride were prepared as follows: 0.5, 1.0, 1.25, 1.5, 1.75, 2.0, 2.25, 2.5, 3.0, and 4.0 per cent. Ten cages containing 4 or more rats each, previously selected so as to be as nearly comparable as possible, were started and given the various solutions as a sole source of drinking water. Within a few days deaths occurred among the higher levels, and within 10 days no rats receiving 2.5, or more per cent salt were alive. It was interesting to note that at lower levels the amount of water consumed became greater with an increasing amount of salt until a concentration was reached which they refused to drink at all, refraining from drinking until thirst finally compelled them to drink a large quantity at one time causing death in a short time. Farmers often give a similar description of cattle dying apparently from salty waters. It was observed that waters containing 1.5 per cent were often fatal to young rats. Growth stopped, the animals presented an emaciated appearance, the coats became rough, eyes were often sore, presenting a condition resembling ophthalmia, and diarrhea was always

present. An autopsy showed the intestines to be inflamed and, in severe cases, bloody. In many cases, spots were found on liver and lungs. The sites of infection, we believe, are due to a secondary cause brought about by the general weakened condition. It was later demonstrated that when the animals on levels up to 1.5 per cent salt survived the preliminary stunted period, they resumed growth and in many cases reproduced and reared their young in a manner somewhat comparable to normal rats. A few survived at higher levels, although the ability to nourish young was very limited. Occasionally, normal young were born, but they often died seemingly due to a lack of action of the mammary glands. Therefore, it is quite apparent that there is some physiological readjustment which should explain how man and animals reared in certain sections thrive on waters that kill or injure those accustomed to the drinking of soft waters only. It was also observed in the course of the investigation that old animals were more resistant to these changes than young. Rats four weeks of age and weighing 50 grams, when placed on 1.5 per cent salt nearly always died. The ones surviving passed through a stunted period, in time started growing, and in some cases grew normally. Full grown animals lost weight but rarely died. We believe this explains why a herd of cattle shipped into these regions will die or be seriously injured, while other animals accustomed to these conditions will live in apparent good health. Likewise, aquatic fowls will rear young on saline lakes, while migrating birds from clear water regions will die by the hundreds in such lakes. The writer's attention has been called to fish living in salt water wastes of oil wells, this water containing over 1 per cent of sodium chloride alone as well as other salts. Yet, fish from surrounding streams would have died if placed in such concentrations.

In working with plant nutritive solutions, it has been found that at a certain level sodium chloride becomes toxic. To this concentration calcium salts could be added, and despite the increased concentration the plant could live. This is explained on the basis of antagonistic action of ions. In order to determine whether this would be true for animals, other salts were added to the dangerous levels of sodium chloride. The additional salt made the previous toxic salt more toxic. Evidently, either antagonistic ion action is not effective for animals, or some other factor predominates in the final results. Animals are also different in that they can use much greater concentrations of salts than plants. All of our observations lead us to believe that there is an osmotic rather than a toxic reaction of salts. The accompanying table shows the quantities of the various salts used, the number of test animals, the effect upon reproduction, the number of

TABLE I

RESULTS OBTAINED BY USE OF SALINE WATERS AS A SOLE SOURCE OF DRINKING SUPPLY FOR RATS

Salt	Per cent	Males	Females	Litters	Young born	Young lived	Young died	Remarks
NaCl	0.5	2	3	3	24	20	4	Growth normal.
	1.0	1	1	1	7	4	3	Growth normal.
	1.5	3	5	5	39	31	8	Growth subnormal. Some die.
	2.0	2	3	3	20	17	3	Young die.
	2.2	1	2	0	0	0	0	Old and young die.
	2.5	4	6	0	0	0	0	Old and young die.
	3.0	2	2	0	0	0	0	Sudden loss in wt., diarrhea, rough hair, and death.
	3.5	2	2	0	0	0	0	Young and mature die soon.
	4.5	4	2	0	0	0	0	Die at once.
NaCl+ KCl	1.5 0.7	2	3	3	39	0	39	Young die within 15 days.
	1.7 0.6	2	2	0	0	0	0	Old ones die.
	2.5 1.0	2	4	0	0	0	0	Old and young die.
	3.0 1.5	2	4	0	0	0	0	All die within a week.
NaCl+ $CaCl_2$	1.5 0.7	5	4	1	7	0	7	Hair thin and rough. No gain.
	3.0 1.5	4	4	0	0	0	0	All die at once.
$CaCl_2$	1.0	2	2	2	13	10	3	General condition good.
	1.5	3	6	7	77	62	15	Growth satisfactory. Condition good.
	2.0	1	2	5	30	6	24	Interferes with lactation.
	2.5	1	1	0	0	0	0	Old ones die.
$MgSO_4$	0.5	2	2	2	10	10	0	Growth and reproduction.
	1.0	1	3	6	51	24	27	Young poor; old emaciated.
	1.5	3	3	3	20	13	7	Growth subnormal; diarrhea, rough coat.
$MgCl_2$	1.0	2	4	2	15	1	14	Condition of old not normal.
	1.5	1	2	0	0	0	0	Growth below normal.
$MgCl_2$+ $CaCl_2$	1.0 0.5	2	2	1	7	0	7	Growth of old normal. Lactation poor.

TABLE I (*Continued*)

Salt	Per cent	Males	Females	Litters	Young born	Young lived	Young died	Remarks
MgAc	1.5	2	2	2	17	8	9	Growth satisfactory. Reproduction.
Na_2CO_3	1.0	3	7	7	45	31	24	Growth of offspring unsatisfactory.
	1.5	1	7	2	19	0	19	Young die; old rough coated, red eyes, diarrhea.
$NaHCO_3$	1.5	2	4	7	55	48	7	Old somewhat undersize. Growth of offspring impeded.
	2.0	1	2	2	14	7	7	Growth and appearance unsatisfactory.
NaOH	0.5	2	4	0	0	0	0	Practically normal growth.
	1.0	3	4	0	0	0	0	Little growth, animals dirty, very nervous, diarrhea, sore eyes.
$Ca(OH)_2$	sat.	4	4	4	30	19	11	Some interference with growth during lactation. 3 generations produce.
$CaSO_4$	sat.	3	8	7	33	28	5	Satisfactory growth.
Na_2SO_3	1.5	1	2	1	11	6	5	Appearance of adults satisfactory.
NaF	1.0	1	2	0	0	0	0	Growth poor. Animals emaciated.
KI	1.0	1	2	0	0	0	0	Growth fair. No reproduction.

young reared, and the general condition of test animals. It also illustrates the results of introducing a mixture of salts into drinking waters. It should be noted at this point that these results have been checked and rechecked over a period of four years. Whenever reproduction occurred, the young were continued on their mother's diet through five or six generations.

The effect of seasonal variation was also given consideration. As one might predict, the hot dry days of summer seemed to be most fatal to animals drinking saline waters, a result undoubtedly due to a greater thirst and therefore greater salt intake.

In streams contaminated by oil well wastes, calcium chloride and

magnesium chloride are often found in large quantities. Examination of these wastes entering streams often shows a salt content of over 20.0 per cent. In many sections near the gypsum beds, waters saturated with calcium sulfate are common; while sodium carbonate and bicarbonate frequently occur in several western states. For these reasons these salts were studied separately and in combinations, as is shown in the table. Calcium chloride is not normally found in most waters, but it is one of the common salts in oil well wastes. Streams and pools in the neighborhood of oil fields are often heavily saturated with this salt. A series of experiments as conducted with sodium chloride were carried out, using calcium chloride as dissolved salt. It was expected that more deleterious effects would be observed, but the contrary was established. An inspection of the table will reveal that at 1.5 per cent, rats, after the first rather difficult adjustment period, grew normally, reproduced their young, and were able to suckle them through the lactating period. In fact, the general appearance, coat, eyes, and build of the rats seemed superior to those of the breeding colony. All of our tests indicated that calcium chloride might prove to be a more satisfactory source of calcium in mineral mixtures than calcium carbonate. Even at 2.0 per cent, growth after the introductory period was normal, but lactation was hindered. At higher levels, results similar to those with sodium chloride were encountered.

Waters saturated with $CaSO_4 \cdot 2H_2O$ are common about gypsum deposits. However, this salt is only soluble to the extent of 0.24 per cent. Waters of this type have not proved to interfere with normal growth, and reproduction.

Magnesium, the third ion quite commonly found in salt well wastes in the form of magnesium chloride, and in many well waters near sulfate deposits as magnesium sulfate, was used in the form of magnesium chloride, sulfate, and acetate. An examination of the tables will demonstrate that magnesium is not so satisfactorily used as either sodium or calcium, but the true condition can only be appreciated by observation of the living animals. When the animals were first given these solutions there was a rapid loss of weight and severe diarrhea; and even in cases where the animals recovered, they did not have smooth coats and a general emaciated condition was presented. It is evident that magnesium in company with the sulfate radical is more detrimental than magnesium chloride and much more so than the acetate.

In many sections of the west, alkali waters containing Na_2CO_3, $NaHCO_3$ and even NaOH are to be found. We have had occasion to analyze samples which contain large quantities of $Ca(OH)_2$. The latter is only soluble to

the extent of 0.18 per cent, but it was rather surprising to find that this hydroxide could be used as a sole source of drinking water through four generations without any evident deleterious results with the exception of a somewhat greater mortality of the young. Sodium hydroxide to the extent of 0.5 per cent permitted life and growth, but 1.0 per cent allowed no growth; the animals became thin and very highly nervous and irritable, the eyes became sore and diarrhea followed. A similar condition was likewise produced by 1.5 per cent of Na_2CO_3; 1.0 per cent of the latter did permit growth and reproduction, but there was a high mortality of young. Sodium hydrogen carbonate, 1.5 per cent, permitted growth, reproduction, and rearing of young. These young, as well as the adult rats, were somewhat undersized. At 2.0 per cent the general appearance became unsatisfactory. With the sodium hydroxide and carbonate we found indications of factors other than osmotic pressure producing their effect; however, it was rather surprising to note the amount of alkali that could be utilized. A number of other salts have been used to answer certain specific cases called to our attention, but as they are not of general interest no comment need be added to the facts presented in the table.

The question has been raised whether or not data obtained from rats would be applicable to other animals. We have made sufficient isolated tests to furnish evidence that they are, at least for cattle, sheep, and rabbits. Other tests now under way will determine this conclusively and should it be proved that larger animals are exceptions to the rule, that fact will be reported at a later date.

Several series of very definite chemical analyses and measurements are also being run to answer definitely certain questions postulated in this article, namely, in regard to the change in osmotic effect, changes in pH of blood stream, and the effect on the organ size. A detailed description of the methods used and results will be forthcoming.

Conclusions

1. Waters containing large amounts of salts in solution are deleterious for drinking purposes.

2. The effect produced seems to be more osmotic in reaction than due to any specific ion.

3. From 1.5 to 1.7 per cent seems to be the maximum amount of soluble salts that can be used with safety.

4. Animals seem much less susceptible to salt solutions than plants, and antagonistic effect of ions is lacking or is a secondary factor.

5. Chloride salts are less injurious than sulfates, and organic salts are less injurious than inorganic.

6. Alkalies are more deleterious than normal salts; evidently the osmotic effect is coupled to a harmful effect of the changed pH.

7. An interference in lactation and reproduction is noticeable even before the level producing stunted growth or death is reached.

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Editorial Review*

THE INFLUENCE OF DIET ON RENAL AND BLOOD VESSEL CHANGES

COMPARATIVELY little of a positive nature is known concerning the effects of diet upon blood vessel and kidney changes, and in general diet is given secondary consideration or is discounted as a factor in the etiology of renal and cardiovascular diseases. Diet experimentation with laboratory animals has failed to supply the keynote to the solution of the clinical problems since species difference introduces a factor of unknown dimensions, but it has produced results of a nature to leave even the most skeptical uncomfortably in doubt. The wide scope of the subject, ranging from the more chemical aspects of nutrition to the highly specialized field of histopathology, adds to the difficulty of investigation. A study which is admirably presented from a histologic standpoint may leave much to be desired from a nutritional point of view. Certain important contributions arising from vitamin studies confine the pathological observations to descriptions of the gross. In considering evidence as conflicting as that associated with the problem under discussion, the reviewer has two alternatives: He may confine his efforts to cataloguing the evidence, a safe procedure but one producing a disjointed product, or he may attempt to evaluate and compare the work, often seeking for common conditions in apparently unrelated environments and for common causal factors in experiments involving uncontrolled variables. Before attempting the present review, tables of experimental data recorded in the literature were prepared, including such headings as number, age, and kind of animals, diet period, diet constituents, blood and urine chemistry, histopathology of kidneys, blood vessels, etc. The panorama of isolated facts and uncontrolled variables so produced struck home very forcibly the dangers encountered in correlation and the unwarranted deductions presented on many occasions.

Cholesterol, protein, acid ash, and vitamins (overdosage as well as deficiency) have been assigned a rôle in the production of kidney and blood vessel changes. For the sake of presentation, these four diet constituents will be considered separately, although it is obviously impossible to consider any one factor without including the others. The renal and arterial changes attending disturbances in purine metabolism (gout), which theoretically might be influenced by diet, can not be discussed because of

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the lack of experimentation and the sparsity of clinical observation. Tartrate nephritis and the sclerosis assigned to alcoholism, while properly included in the subject under discussion, will not be considered, as they represent isolated cases. Furthermore, no attempt will be made to discuss the extensive literature on the spontaneous occurrence of kidney and blood vessel changes in laboratory animals, studies which are obviously fundamental to an understanding of the changes produced by experimentation. Judgment should rest on statistics based on the incidence of renal changes in control and experimental groups and not on opinion as to whether changes observed at the termination of an experiment might be accounted for by spontaneous development. Unfortunately, some useless discussion has arisen concerning the supposed obstacle of spontaneous change which is more or less inherent in all biological research. In the present instance the etiology of spontaneous changes is not known. Infection is the most likely factor. In some of the early diet experiments aimed to produce kidney and blood vessel changes there was an obvious deficiency of vitamins, which led to a lowered resistance to bacterial invasion. The kidney and blood vessel diseases produced in experiments of this type were in some cases suspiciously like the spontaneous type of changes. For this reason especially, attempts to produce renal and blood vessel changes by diet must carry the assurance of adequate vitamin control before they may be seriously considered.

* * *

Since vitamin A deficiency frequently causes changes in epithelial tissue, lesions of the kidney epithelium might be expected on diets deficient in vitamin A. van Leersum (100) has demonstrated the effect in rats by histological studies. Epithelial cells impregnated with calcium were found in the lumen of the tubules. Osborne and Mendel (78) had previously observed calculosis in the bladders of rats on diets deficient in vitamin A. Vitamin A deficiency probably played no part in the nephrotoxic effect of the more carefully designed experimental diets, which included adequate amounts of this substance. The calcium deposits in the tubules of the kidney described by van Leersum (100) are similar to the changes produced in the rat by the fat-free diets of Burr and Burr (15, 19). It is unlikely that any of the nephrotoxic diets employed in the past in rat experimentation were sufficiently fat-free to produce the deficiency disease described by Burr and Burr. In the discussion to follow it becomes apparent that vitamin deficiency was often present in conditions which led to kidney and blood vessel changes, but it is generally impossible to decide which known vitamin or vitamins may have been lacking.

* * *

It is now established by the work of Yuasa (102) and of Schönheimer (89) that changes in the intima of the aorta and other blood vessels indistinguishable from the lesions observed in human arteriosclerosis may be produced in herbivorous or carnivorous animals by the addition of cholesterol to apparently adequate diets. The early work has been reviewed by MacCallum (52). Experimental proof was lacking in many of the earlier investigations because natural foodstuffs containing cholesterol instead of the purified sterol were used in the experimental diet. Since these natural foodstuffs were in many instances also high in protein, protein was cited as a possible causal factor. Later, when it was shown that cholesterol alone could be responsible for the changes produced, the question arose as to whether the arteriosclerosis produced in certain high protein feeding experiments could not be accounted for by the cholesterol content instead of the protein. Clarkson and Newburgh (23) showed in working with rabbits that when cholesterol was added to their control diet in amounts equalling the cholesterol content of their beef muscle diet, which produced arterial changes indistinguishable from the "cholesterol" sclerosis in rabbits, no changes were observed even though the duration of the feeding exceeded the time limit in which changes were produced by the high protein diet. It was only by increasing the added cholesterol tenfold that the characteristic lesions appeared. Anitschkow (5), however, fed rabbits only a few milligrams of cholesterol[1] (as milk) daily over a long period (2.5 years) and produced marked sclerosis, while Kon[2] failed to produce sclerosis in rabbits on a liver diet freed from cholesterol. It remains a fact that arteriosclerosis has never been produced in experimental animals by cholesterol-free diets. The arteriopathic, supposedly cholesterol-free, oats diet employed by Nuzum *et al.* (76) was augmented with cod liver oil, and the casein diet of Newburgh (69), which produced changes similar to those described by Nuzum, contained milk. The evidence at hand indicates that cholesterol might be the predisposing factor in all experiments in which arteriosclerosis has been produced; the cholesterol feeding experiments of Newburgh might indicate that the amounts necessary to produce the lesions are not independent of other diet factors. It should be noted that the control diet employed by Newburgh was markedly different from the arteriopathic diet in constituents other than cholesterol.

The significance of the ease with which cholesterol sclerosis is produced in the herbivorous rabbit in relation to the etiology of human arterio-

[1] The original paper gives no data for estimating the cholesterol ingested. The quotations of later reviewers may be based upon private communications.

[2] This reference was not found.

sclerosis is diminished by the results obtained in experiments with omnivorous animals. In these, arterial changes are produced only by massive doses and secondarily to a cholesterolemia and a large accumulation of lipoid material in the other organs of the body, especially in the liver, a condition not characteristic of human sclerosis. Moreover, arterial or glomerular changes in the kidney comparable to the lesions associated with late human arteriosclerosis have not been produced by feeding excessive amounts of cholesterol. In the rabbit the kidney becomes fatty, but the changes resemble more nearly those in the spontaneous nephritis of rabbits. Evidence of a hypercholesterolemia in humans suffering with hypertension and arteriosclerosis has been sought, Pribram and Klein (82), Mjassnikow (63), and Labbé and Heitz (43) reporting positive results. Characteristic abnormalities in the blood lipids of patients suffering with these diseases were not found by Bloor (14), by Denis (28), or by Cantieri (21). The negative evidence must be given preference since a variety of abnormalities in the blood of arteriosclerotics has been reported, none of which is, however, characteristic. Negative evidence by no means settles the question. Bloor believes that the deposition of cholesterol and its esters does not necessarily require a high blood cholesterol, but that it depends on the ability of the blood to keep in solution a substance which is probably in a state of supersaturation.

The Virchow-Aschoff theory of the production of human arteriosclerosis through cholesterol deposition derives its greatest support from studies on diabetics, in whom the incidence of arteriosclerosis is unusually high and in whom lipemia is a characteristic condition in untreated cases (41). The lipemia of diabetes cannot be ascribed to fat feeding. Joslin has reviewed the cases described in the literature, where on improvement with high fat diets marked lipemias fell to normal levels. Insulin therapy has increased the death rate due to arteriosclerosis presumably by decreasing the deaths from coma. If hypercholesterolemia is the predisposing factor, insulin therapy would supposedly check the sclerosis in regulating fat metabolism by its established action in abolishing hypercholesterolemia. Bowen and Koenig (16) believe that neglect in treatment of diabetes leads to sclerosis. A complicating factor in studying the problem has been the use of high fat diets, incidentally high in cholesterol, in the treatment of diabetes. The widespread use of high carbohydrate diets as advocated by Sansum *et al.* (87) and by Adlersberg and Porges (3) will eventually furnish statistical data of a decisive nature. Joslin (41) compared the blood cholesterol values of groups of diabetics with either slight or marked arteriosclerosis with the values of a group free from sclerosis. The latter

group showed the higher values! Since the etiology of diabetes is of an unknown nature, and since the diabetic of the past was forced to subsist on an excessively high fat diet, the evidence gathered from the study of diabetes that a derangement in cholesterol metabolism might be responsible for arteriosclerosis loses force when applied to the non-diabetic arteriosclerotic. It has been impossible from statistical studies on the incidence of arteriosclerosis in meat-eating and non-meat-eating peoples to correlate cholesterol intake with incidence of arteriosclerosis, because those who do not eat meat usually consume large quantities of milk and eggs.

Because of the similarity in structure between cholesterol and vitamin D, the experiments on vitamin D overdosage, which result in marked histologic changes of the kidney and blood vessels accompanied by albuminuria and nitrogen retention, are of interest. The process is regarded as one of calcification. Calcium deposits have been observed in the aorta, (97a) and in the experiments of Light, Miller and Frey (48) the ash content of the kidney was increased sevenfold after vitamin D overdosage. These workers observed that the overdosage in the first and second generations produced striking pathological changes in the third and fourth generations. Moreau *et al.* (65) believe that the arterial changes resemble those of human arteriosclerosis, while Herzenberg (36) can see no similarity. A compromise is struck in the interpretation of Spies and Glover (94):

> The composite histological changes (rabbit kidney) were strikingly different from the picture of any known pathological process occurring in man. Nevertheless, after decalcification, the lesions in the glomerular arterioles and the interlobular arteries did resemble the renal lesions associated with hypertension.

Schönheimer (89) has built up a very convincing picture of sterol metabolism, of particular interest in relation to the arterial changes which may be produced by cholesterol and vitamin D. He has shown that with the exception of irradiated ergosterol the phytosterols are not absorbed to any extent through the gastro-intestinal tract of either herbivorous or omnivorous animals, and that the presence of cholesterol in the body of the herbivorous animal and to some extent of the omnivorous animal must be accounted for by endogenous reactions. He has suggested that cholesterol is the starting point for an auto-oxidation reduction in which ergosterol and dihydrocholesterol are the end products.

* * *

That kidney damage might result from the long continued excretion of excessive amounts of the end products of protein metabolism is not an unreasonable postulate, even on purely theoretical grounds. In 1919,

Newburgh (69) reported the production of nephritis in rabbits fed diets excessively high in protein, precipitating a controversy which is to-day not entirely settled. A review through 1928 by Mitchell appeared in an editorial in this Journal (62) and no attempt will be made to discuss all the experiments of that period. In general the criticism launched against experiments of a positive nature was the failure to demonstrate the presence of the nutritive factors other than protein essential to normal body development and function. Much discussion arose as to the identity of the histological changes observed with spontaneous changes known to occur, and as to the interpretation of histological findings.

Casein has been the protein most often employed in making up quasi synthetic diets for high protein feeding experiments. In 1925, Evans and Risley (31) reported tubular and glomerular kidney changes in rats fed high casein diets. Osborne and Mendel (78) had previously maintained rats on high casein diets for periods covering the seven-month period studied by Evans and Risley without producing kidney change. In 1926, Jackson and Riggs (40), Smith and Jones (91), and Addis and the MacKays (1), employing casein as the protein element of the diet, all reported negative results. The following year, Smith, Moise, and Jones (92), working with unilaterally nephrectomized animals, succeeded in producing tubular changes. In 1928, Newburgh and Curtis (71) compared the effects of a casein, a liver, and a muscle diet, and showed that kidney changes occurred more slowly in the casein than in the muscle and liver series, the minimum time set for casein being a year. On the basis of these results, the negative findings of certain of the earlier workers were invalidated, but the failure of Jackson and Riggs (40), who carried some of their experiments through eighteen months, to produce changes was still unexplained. At this time Moise and Smith (64), and Smith, Moise and Jones (92), showed that the age of the animal was a factor which had not been considered. Working with unilaterally nephrectomized animals, they found that no kidney changes were produced when the animals were placed on the experimental diets at thirty days of age for a period extending to 280 days, but that when the experiment was initiated on 120 to 200 day old rats, changes resembling the focal lesions that are found in old rats developed. In experiments of a similar nature, Jackson and Moore (39) obtained severe nephritis in four out of eleven rats. In the latest report of the Yale workers (79), gestation and lactation were superimposed upon the unilaterally nephrectomized rats. It was found that the diet employed by Smith, Moise and Jones (92) was not adequate for the production of good litters. More complex diets were instituted. Renal lesions were ob-

served in a few of the rats that were subjected to the greatest reproductive strain on a high casein diet in a three to six-month period. Barring experiments on very old or unilaterally nephrectomized rats, Newburgh and Curtis (71) and Evans and Risley (31) are the only workers reporting severe kidney damage on high casein diets in normal animals. The diets employed by Evans and Risley were obviously deficient in the elements necessary for normal growth. The question arises whether the diet employed by Newburgh, which was adequate for growth, did not lack other requisites for which there was no objective measure. The Newburgh diet contained 75 per cent casein, 16 per cent lard, 1 per cent salt, 3 per cent cod liver oil. Fifty milligrams of a yeast extract were given daily.

In summing up the work (on high casein diets), it may be concluded that on a high casein diet containing the elements essential to growth and normal function, rats are able to survive for periods covering the greater part of the normal life span without suffering renal lesions characteristic of nephritis.

Renal and arterial changes associated with the clinical pictures of nephritis and hypertension have been more readily produced by the use of diets containing natural foods than by those of a more synthetic nature. The nephropathic diets containing egg white, or soya beans, used in the original rabbit experiment of Newburgh (69), were obviously deficient in vitamin and salt content. The soya bean and peanut diets used by Evans and Risley were of the same category. The beef muscle and liver diets of Newburgh (71), used in his rat experiments, were augmented with salts, cod liver oil and yeast extract. Maclean *et al.* (55) believe that the kidney changes produced by the latter diet can be attributed to a lack of greens in the diet. These authors showed that marked tubular damage of the kidneys of rabbits fed on either high or low protein was produced in a month's time. The effect was abolished by the addition of a "small" amount of greens daily. Unfortunately the normal kidneys were obtained from animals fed on the greens diet only five to twelve months. A period of two years was required by Nuzum, Osborne, and Sansum (76) to produce both arterial and tubular and glomerular changes in rabbits on liver and oats diets augmented with cod liver oil and salts, and with greens given twice weekly. The beef protein diets of Newburgh (70) used in his rabbit series were augmented with 100 gm. of greens weekly. A discrepancy between the results obtained by Anderson (4) and those of Newburgh and Clarkson (70) is a stumbling block to interpretation. The diets employed by both workers were very similar. Beef and flour constituted the bulk of the diets, Newburgh's being augmented with 100 gm. of greens, and

Anderson's with 10 per cent alfalfa, and lettuce given once a week. Anderson superimposed a partial removal of kidney substance. No kidney damage was observed by Anderson while profound changes were reported by Newburgh. The Santa Barbara workers fed rabbits on barley for a 16 to 23-month period. The animals showed marked symptoms of diet deficiency, finally succumbing. Alfalfa was fed, 20 gm. weekly, after the first six months of the experimental period. No kidney or blood vessel changes were noted. The experiments by Maclean (55) have been cited so extensively that it seems fitting to consider them in some detail. Their low (12 per cent) protein diet consisted of bran and oats. Their high (60 per cent) protein diet contained bran, casein, gelatin and salt. Both of these diets produced tubular lesion in less than two months. The addition of "a little green food" entirely prevented the occurrence of kidney damage. While the actual amount of green food is not given, it transpires that two leaves of cabbage a day were fed each rabbit. It is also noted that the reaction of the urine on the cabbage diet was with one exception alkaline or neutral, while that of the greens-free diet was acid. Since rabbit kidneys do not compensate to any great extent in the formation of ammonia when acid urine is being produced, it is difficult to understand how the daily ingestion of only two leaves of cabbage shifted the reaction of the urine so markedly. Maclean's work undoubtedly showed that the tubular lesions which developed in the rabbits on his particular greens-free diet cannot be traced to the dietary protein.

Since high casein synthetic diets produce kidney lesions only under exceptional strain, and certain natural food protein diets such as liver produce renal damage in a comparatively short time, it is more or less evident that if protein is the predisposing factor, the nephropathic property is not common to all proteins. Mitchell (62) has emphasized that the production of renal changes might just as well be attributed to the non-protein constituents of the natural protein foods. Newburgh and Johnston (73) recently described experiments corroborating these views. They found that high protein feeding of lactalbumin, and of wheat and soya bean glutens, augmented with vitamins and salts, produced no renal changes in a year's time, while a 40 per cent liver diet, or a 20 per cent sodium nucleate diet, was nephrotoxic in less than a year. The nucleic acid was prepared from liver, and when ingested in the amounts contained in a nephropathic liver diet was insufficient to produce renal changes. The nephropathy produced by nucleic acid was characterized by formation of scar tissue and by thickening of the arteriole walls, a picture not the same as that produced by liver feeding. Newburgh concluded that liver may contain

nephrotoxic forms of non-protein nitrogen other than purines. The deduction is not clear since the nucleic acid was presumably bound in a protein molecule in the liver, and would consequently be considered an intermediary product of protein metabolism. The recent preliminary report of Blatherwick *et al.* (13) on the effect of liver and beef muscle diets on unilaterally nephrectomized rats will leave no doubt to even the most skeptical histologist that lesions of great severity, very similar to chronic glomerulonephritis in man, may be produced by feeding experiments. Blatherwick supplemented his diets with yeast, cod liver oil, lard and salts, and also greens, so that the objections raised to older work are met. Blatherwick, like Newburgh, also studied a liver residue fraction with the idea of finding what fraction of liver contains the nephrotoxic substances.

The production of renal injuries by the intravenous or oral administration of certain amino-acids, notably cystine, has been cited as evidence that high protein feeding is one of the factors in the etiology of nephritis. An obvious objection to experiments in which cystine was fed as an amino-acid is that free cystine would not suffer the same ultimate fate as protein-bound cystine because of the difference in the rate of alimentary absorption. Curtis, Newburgh, and Thomas (27) established the amounts of cystine which would injure the rat kidney. A diet containing 1.5 per cent cystine produced necrosis of the tubules in one year, while 5 per cent cystine was lethal in a few weeks. Evidence has recently been presented by Cox which indicates that, at least as far as young animals are concerned, vitamin B requirement and cystine intake are closely related. Cox and Hudson (25) showed that the addition of large amounts of yeast to a 0.3 per cent cystine diet, which was nephrotoxic to young rats, prevented the renal injury entirely. It seems worth mentioning that the 1 per cent cystine diet fed rats by Addis (1), without production of renal changes, contained 10 per cent yeast. Unfortunately Addis stopped his experiments at twelve months, the minimum time Newburgh found necessary to produce lesions of the kidney on a diet containing approximately the same amount of cystine, so that it remains to be shown whether or not the protective effect of yeast against the nephropathic action of cystine is the same in young and old animals. When Hartwell (34) substituted edestin for casein in feeding experiments on young rats, the animals died showing kidney changes, unless vitamin B content was greatly augmented. The protective factor was not destroyed by autoclaving. Older rats were not affected by the diet low in vitamin B. Hartwell concluded that edestin requires more yeast than either caseinogen or egg albumin for normal metabolism. Unfortunately a histological report of the pathological kidneys described by

Hartwell is not available. The experiments of Cox and of Hartwell indicate that the minimal vitamin B requirement is dependent on the nature of the other diet constituents. If this postulate be accepted, the interpretation of all diet experiments concerned with renal changes is complicated by the uncertainty of the vitamin B requirement, even the recent work of Newburgh (73) and of Blatherwick (13). Francis, Smith, and Moise (32) have recently shown that the renal hypertrophy caused by the feeding of high protein diets is not affected by vitamin feeding. Their experiments did not produce kidney damage. There is at present no evidence that the excretion of any of the known amino-acids in the human is responsible for renal injury. Excepting conditions in which there is severe liver damage, the amino-acid concentration of the blood never rises markedly and relatively small amounts of amino-acids appear in the urine.

Realizing the inherent difficulties involved in designing a diet experiment for laboratory animals which will produce decisive evidence, one approaches the human experiment with great temerity. Following the results of high protein feeding on animals, Squier and Newburgh (95) studied the effect of high protein feeding in normal humans and in several cases of essential hypertension. When two normal men ate three pounds of steak a day for several days, red cells but no albumin appeared in the urine. In five cases of essential hypertension, the ingestion of 100 to 175 gm. of protein per day over a period of two to twenty-one days was accompanied by the appearance of red cells and albumin in the urine. It is now generally recognized that the appearance of red cells in the urine under the experimental conditions of the Squier-Newburgh experiment is without significance. McClellan and DuBois (50) studied the effect of a meat diet containing 100 to 140 gm. of protein and 200 to 300 gm. of fat, which was followed for a year. No evidence of kidney damage was obtained. Nitrogen equilibrium was maintained on a daily intake of 19 gm. Newburgh, Falcon-Lesses, and Johnston (72) repeated the high protein experiment, using a diet containing 337 gm. of protein per day, 31 per cent of the caloric intake being in protein. Albumin and casts appeared in the urine of a normal individual who followed this diet for a six-month period. On resuming a normal diet, the albumin and casts disappeared. It is controversial whether the appearance of a slight amount of albumin and a few casts is indicative of renal damage. In the rabbit, the appearance of albumin follows uniformly the excretion of an acid urine. In the experiments of Bischoff (12) no histological renal changes were found in rabbits which had passed an acid urine and shown traces of albumin for nearly two years. The albuminuria produced in rabbits by the passage of

an acid urine has been explained on the basis of a change in cell permeability.[3] The Newburgh diet (72), as far as protein content is concerned, approached more nearly the diet of the Eskimo near civilization while that of McClellan and DuBois (50) was similar to the diet of the Eskimo away from civilization. The Eskimo, subsisting on a carnivorous diet, which according to Krogh averages 280 gm. of protein daily, ingests excessive amounts of cholesterol, protein, and acid ash, and apparently tolerates these supposedly harmful substances well, for the incidence of renal-cardiovascular diseases according to Thomas (99) is low.

Newburgh (72), however, is inclined to discount the conclusions of Thomas, based on a survey of 142 Greenland Eskimos, that an 8.5 per cent incidence of albuminuria was not indicative of prevalent renal disorders. He cites the statistics on over 16,000 policy-holders of the Metropolitan Life Insurance Company. The comparison is hardly justifiable since policy-holders are picked. Moreover, the age limit of the group of policy-holders selected was five years below that of the group of Eskimos. Furthermore, the difference in incidence of albuminuria for the two groups is less than three times the standard deviation of the mean. The discrepancy in results obtained in the Newburgh experiment, in which albuminuria was produced, and the findings in the studies on the Eskimo, who does not normally show albuminuria, has not been explained. The Newburgh diet contained 80 gm. of liver a day. It is regrettable that this substance, which according to Newburgh's own animal experiments has a specific nephrotoxic effect, was included in the diet.

The experiments of McClellan and DuBois indicate that amounts of protein which would be considered high in the diet of civilized man may be eaten for a comparatively long period without suggestion of renal injury. The Newburgh experiments indicate that the tolerance of the human kidney to certain high protein food metabolites is not without limit.

It should be emphasized that Newburgh (72, 95) noted no effect of high protein feeding on blood pressure, an observation in agreement with the reports of Strouse and Kelman (97) and of others, that variations in blood pressure bear no direct relation to intake of protein. The latter statement is, however, not universally accepted. The low incidence of hypertension among the Chinese has been ascribed to the diet, which is low in protein. Cadbury (20) found that the blood pressure of Chinese was lower than that of Americans or Europeans of the same age. Diet is apparently not the only factor, for according to Houston (37a) most white

[3] Bayer, W., *Z. ges. exper. med.*, 1928, 59, 162.

men have a fall in blood pressure during a prolonged sojourn in China, even on a normal European diet. Saile (85), in comparing monks who subsisted upon an herbivorous diet with those who partook of meat, found a lower blood pressure range in the group on the vegetarian diet. It should be noted that the vegetarian monks partook of only bread and water on their weekly fast day and during their forty-day fast period. In contrast to the meat-eating groups, the vegetarian monks were allowed very little liquor. Underfeeding has been shown by Benedict *et al.* (11) to lower blood pressure. The subjects observed by Cadbury (20) and by Saile (85) may have been undernourished. Mosenthal (66) originally suggested the latter explanation to account for the impression that low protein diets lower blood pressure.

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Since many of the synthetic and natural food high protein diets, associated with the production of renal and blood vessel changes, were necessarily high in "acid ash," the nature and content of the ash became one of the variables to be considered in seeking the causal factor. Such diets produce an acid urine, and the proof of the theory lies in showing that the degree of acidity in itself, or the burden placed upon the kidney in maintaining the acid base equilibrium of the body, is responsible for the renal changes. In order to show a relation to the production of arteriosclerosis, a demonstration of a shift in the acid base equilibrium of the blood or tissues would be required. Newburgh and Clarkson (70) in their original rabbit experiments attributed the renal injuries produced either to the excessive excretion of amino-acids or to a combined effect of amino-acids and acid urine. They did not consider the acidity of the urine by itself capable of producing all the injury. Nuzum, Osborne and Sansum (76), using more complete diets and instituting a series of controls, confirmed the work of Newburgh (70), but hesitated to ascribe the results to the protein *per se*. Since they observed a decrease in the CO_2 combining power of the blood plasma of rabbits maintained on oats or liver-containing diets, they had produced evidence that acidosis might be a predisposing factor. In later experiments, however, Nuzum *et al.* (77) failed to reproduce the fall in CO_2 combining power on the same diet used in the earlier experiments in spite of the fact that animals with a spontaneous nephritis were used. The changes observed, other than the fall in CO_2 combining power, confirmed the original work. Bischoff, Sansum, Long, and Evans (12), using a diet of the same acid ash content as that employed by Nuzum and instituting a more refined technic in the determination of acid base changes, failed to observe changes in plasma pH and CO_2 content, or

damage to the blood vessels or kidneys of rabbits. Nuzum and Newburgh both have produced by an alkaline ash soya bean diet, kidney changes similar to those produced by acid ash diets. The original nephropathic casein diet of Newburgh was potentially neutral in reaction. It would therefore appear that in the rabbit the association of acid ash with kidney and blood vessel changes was fortuitous.

The kidney of the rat has such a remarkable compensating mechanism in the formation of ammonia when the urinary constituents are predominantly acid in nature, that acid urines down to the danger point of pH 4.8 can not be produced by feeding acid ash foodstuffs. The lowest value found by Long and Sansum (unpublished data) for acid ash foodstuffs was 6.1. In order to eliminate the increased burden inflicted upon the kidney in ammonia formation, Polvogt, McCollum, and Simmonds (81) adjusted their high protein diets so that the ash content was potentially alkaline. They observed renal changes. Addis (1) added calcium chloride to his casein diet so that the urinary pH was 5.2 as compared with 6.4 for his control series. No kidney damage was produced. Recently Blatherwick (13), working with unilaterally nephrectomized rats, produced nephritis by liver feeding. In one series of his experiments, sodium bicarbonate sufficient to neutralize the acid ash of the liver was added. The kidney damage, if anything, was greater on the neutral ash diet. The conclusions recently formulated by Samuel and Kugelmass (86), that acid-forming diets shift the acid base mechanism and inhibit growth, development, and metabolism in young rats, are obviously unwarranted, since the acid base factor was only one of many variables in their two series of experiments. These investigators ignored the work of Green and Mellanby (33) on the interfering effect of cereals on calcification, although their acid ash diet consisted of egg, rice and oatmeal. Their basic diet contained dried milk, potato, and beans.

In man the acid or alkali content of ingested foodstuffs is insufficient to produce a sustained shift of the acid base equilibrium outside the limits of normal. Michalowsky (60) observed no changes in the alkali reserve on a mixed diet of meat, fat, and wheat. Unpublished data of Bischoff, Long and Sansum show that the daily ingestion by an arteriosclerotic patient of sodium citrate equivalent to 18 pounds of oranges for a period of three months, shifted the pre-breakfast plasma pH and total CO_2 of the blood just significantly outside the individual's control range, but not outside the normal group limits. The ingestion for a three-day period of protein foods containing an acid ash equivalent of 2.5 pounds of beefsteak failed to produce any effect on the acid base equilibrium of the blood, although

there was marked nitrogen retention. The urinary pH failed to become more acid than 5.0 due to the increased formation of ammonia. Previously Michalowsky (60) had shown that the ingestion of 2.5 gm. of phosphoric acid daily (equivalent in acid ash to less than one pound lean beef) for a period of six weeks had no effect upon the alkali reserve. It is likely that the ingestion of natural foodstuffs produces temporary changes in the acid base equilibrium of the blood, but it is doubtful whether these changes are as great as those produced by exercise, and, therefore, whether they are significant. Experimentation is complicated by the "alkaline" tide and awaits investigation.

While there is at present little evidence that the acid ash foods produce kidney or blood vessel changes by virtue of a shift in the acid base equilibrium of the body, it is possible that the character of the ash constituents, other than potential acidity, is involved in the changes produced. Diets high in the acid ash foods are usually high in phosphorus and low in calcium. The calcium-phosphorus ratio has been adjusted in the nephrophatic diets of a sufficient number of workers (Nuzum (76), McCollum (81), Blatherwick (13)), to eliminate the factor of ratio. A high phosphorus content, irrespective of calcium low or high, has not been controlled. The failure of many workers to produce renal damage in rats on high casein diets and the failure of the Santa Barbara workers to produce renal changes in rabbits on prolonged barley feeding, involving in both cases high phosphorus diets, is evidence of a negative character that phosphorus is not a causal factor. MacKay and Oliver (53) have assigned a positive influence to phosphates in affecting renal hypertrophy and tubular damage. They found that acid, neutral and basic phosphate, all produced the same effect, so that the reaction of the phosphate was eliminated as a factor. Deductions concerning the effect of high phosphorus (high protein) diets based on the feeding of phosphate are analogous to deductions concerning the effect of high protein diets based on the feeding of amino-acids; the liberation of bound phosphorus as phosphate in the catabolism of ingested nutrients would presumably be a more prolonged and uniform process than the absorption of phosphate. Constituents other than calcium or phosphorus in the ash content of the diet have thus far not been considered in feeding experiments concerned with the production of renal or blood vessel changes.

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In summing up the experimental work on the effect of diet on renal and blood vessel changes, it becomes apparent that changes in the blood vessels outside the kidney, and changes in the tubules, glomeruli, and ar-

terioles of the kidney, may be produced by diet constituents, but in no case can any one nutritional entity be held responsible for producing a clinical picture resembling the progressive characteristics of human hypertension followed by arteriosclerosis and by nephritis. Newburgh (70) and Nuzum (76) came close to producing such clinical pictures in rabbit experiments, but three variables at least may have been concerned in producing the changes: cholesterol, specific protein foods, and lack of vitamins. The cholesterol might account for the arterial changes and the protein or associated compounds for the renal injury, while vitamin deficiency with attending bacterial invasion can not be eliminated as a superimposed influence. In rat experiments, no aortic changes were produced by nephrotoxic diets, and no characteristic renal changes by arteriopathic diets. In rats, Blatherwick (13) produced albuminuria with a change in the ratio of the plasma protein by feeding natural protein foods, the very substances used in the treatment of humans with the same findings. The original ideas concerning the three constituents which have been held responsible for kidney or blood vessel changes induced by diet feeding experiments, have not stood the test of time. Sensitivity to cholesterol has been shown to vary largely with species. The ideas concerning the effects of high protein feeding have undergone a series of metamorphoses; the effects ascribed to protein are either specific for a certain kind of protein or are due to non-protein constituents associated with protein foods. Acid ash at present appears to be without significance. In the case of humans,[4] degenerative changes produced by lack of vitamin A in the diet or by an excess of vitamin D in the diet would presumably be regarded as isolated cases. In considering the rôle of diet in the treatment of arteriosclerosis and nephritis, the greatest caution must obviously be exercised in drawing deductions from animal experiments. If man were to eliminate from his diet all the substances which have been held responsible for producing blood vessel and kidney changes in animal experiments, he would become a vegetarian in the strictest sense of the word, denying himself even milk or eggs. Incidentally, in joining the herbivora he would be joining a class with a high incidence of kidney damage.

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The nephritic of twenty years ago was invariably placed upon a low protein diet. Restriction of nitrogen intake was considered desirable to lessen the burden upon the kidney in excreting urea. The practice, however, anteceded this explanation of the last generation. It is possible that the

[4] Harris, L. J., *Annual Rev. Biochem. Stanford*, 1932, **1**, 365.

production of albuminuria was associated with a misconception of a derangement in protein metabolism prompted by the analogy of sugar excretion in diabetes. It is now generally recognized that the nephritic is no exception to the rule that the protein requirements of the organism must be met, and since there is in nephrosis and certain other types of Bright's disease a considerable leakage of protein in the urine, the protein requirement may be greater than that of the normal individual. Epstein (30a) originally advised the use of high protein diets for certain types of nephrosis. Peters and Van Slyke (80) have emphasized the dangers of low protein feeding in nephritis. These authors point out that there is no relation between the amounts of protein fed and the amount excreted in the urine on moderately high protein diets; furthermore adults with the degenerative type of nephritis may apparently be protein-starved, since they have an amazing ability to store nitrogen for long periods without increasing the non-protein nitrogen of the blood or urine. Although the non-protein nitrogen of the blood of patients with advanced nephritis may be reduced by low protein-feeding, Peters and Van Slyke doubt the significance of this finding in relation to the cause of the disease. The clinician is thus faced with a paradoxical situation. Although animal experimentation has shown that high intake of certain protein foods is productive of renal injury, he must discount the probability of increased consumption of nephrotoxic substances for the more urgent requirements of the body as a whole.

In a study of 500 persons, Langstroth (45) found an apparent relation between the percentage of non-protective (vitamin deficient) food which had made up the usual diet of the subject and the incidence of degenerative disease. On the basis of this observation, forty odd patients with chronic circulatory diseases were placed on diets high in protective foods. Marked improvement was noted. A significant fall in blood pressure was observed in 18 out of 23. Langstroth attributed the effect in part to a reduction in caloric intake, a procedure which previously had been found effective by Rose (84) and by Terry (98), but emphasized the importance of a high intake of protective foods. He pointed out that the fall in blood pressure did not in all cases occur independently of relief from distressing symptoms. Langstroth's dietary survey was based on information obtained by questioning the subjects, and is in this respect unique. The results are certainly in harmony with animal experimentation, in which degenerative changes are more easily produced on vitamin-deficient diets. The clinical results reported by Langstroth are typical of those attributed to scores of regimes for the treatment of circulatory diseases, but proof is lacking that the regime affects or even arrests the degenerative processes.

The fundamental difficulty in work of this nature is the lack of acceptable objective measures. A fall in blood pressure unfortunately can not be regarded as convincing, since the blood pressure of the patient with hypertension is notoriously subject to great lability. Ayman (7) recently prescribed "seriously and enthusiastically" to forty unselected hypertensive patients a daily dose of a few drops of very dilute hydrochloric acid and found definite improvement in 82 per cent of the cases. He concluded that the symptoms associated with uncomplicated essential hypertension[5] may frequently be relieved by the suggestion inherent in a prescribed method of treatment. Since the non-protective foods are as a class high in acid ash, the alkaline ash diets of Sansum *et al.* (88), used in the treatment of vascular diseases, are essentially the same as those employed by Langstroth, although the *modus operandi*, the production of a neutral urine, is of course entirely different. A diet along the same lines had previously been described for the treatment of nephritis by Chace and Rose (22). What has been said about the Langstroth diet applies equally well to the diets of Sansum and of Chace. A statistical survey of survival rates of patients with essential hypertension who have followed such diet regimes as compared with patients who were allowed freedom in food selection, would do much to settle the question.

Since a loss of base with an attending acidosis may be a manifestation of terminal nephritis, the use of alkaline ash diets in the treatment of nephritis would appear logical. The objection to these diets is that they are apt to be too low in protein. Nothing is gained in attempting to neutralize the sulfur and phosphorus of protein by the addition of fruits and vegetables high in sodium and potassium, since the work of Shohl and Sato (90), of Briggs (17), and of Bassett, Elden, and McCann (9), show that the elimination of the acidic elements is not enhanced by increase of base. According to Briggs, the virtue of the basic diet lies in the limitation of the phosphoric and sulfuric acids to be excreted and the specific action of calcium in increasing elimination of those acids. While it is recognized

[5] It may be questioned whether essential hypertension is properly included under the heading of kidney and blood vessel changes. Even to-day it is not universally recognized that hypertension generally precedes arteriosclerosis by a considerable lapse of time. In the experiments of Nuzum *et al.* (76, 77) and of Anderson (4) discussed in this review, blood pressure data were collected. Anderson noted no change in the blood pressure of the rabbits which developed arteriosclerosis. Nuzum on the other hand noted a slight elevation in blood pressure for the groups of rabbits which developed arteriosclerosis, but none for a group which showed kidney damage without arteriosclerosis. The production by diet experimentation of arteriosclerosis, the lesion of significance in human pathology unless associated with a preceding hypertension, may be an entirely different process from that taking place in humans.

that the ability of the kidney for ammonia formation is reduced in nephritis, there is disagreement as to whether ammonia formation conserves base or merely protects the kidney from acid. Whatever the mechanism, the basic diet would appear superior to one high in acid ash, provided the protein is adequate. Peters (80), admitting the basis for the use of alkaline ash diets in terminal nephritis as theoretically sound, has pointed out that experimental data are lacking to show the effects of the treatment.

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The survey of animal experiments, of diet habits of various peoples, and of clinical diet procedures, leaves one with the impression that diet may have little to do with the spontaneous kidney and blood vessel changes observed in lower animals and with the cardiovascular renal diseases found in man. The progress of the research is at that stage where positive results are attained only by a degree of exaggeration which destroys their practical significance. A more thorough application of the statistical method and more carefully designed clinical experiments based on acceptable objective measures must be instituted in the study of diet in hypertension, arteriosclerosis, and nephritis. The uncertainty of the effect of diet on blood vessel and renal changes in man is an unwelcome spectre in the sciences of nutrition and medicine.

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